Proteasomal Degradation of Spermidine/Spermine N$^1$-Acetyltransferase Requires the Carboxyl-terminal Glutamic Acid Residues*

(Received for publication, January 17, 1997, and in revised form, February 24 1997)

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The rapid turnover of spermidine/spermine N$^1$-acetyltransferase (SSAT), a key enzyme in the regulation of polyamine levels, was found to be mediated via ubiquitination and the proteasomal system. SSAT degradation was blocked by the binding of polyamines or of the polyamine analog, N$^1$,N$^{12}$-bis(ethyl)spermine (BE-3-4-3), to the protein, providing a mechanism for the increase of SSAT activity in response to these agents. Site-directed mutagenesis indicated that a number of residues including arginine 19, cysteine 122, histidine 126, glutamic acid 152, arginine 155, and methionine 167 were needed for protection of SSAT by BE-3-4-3. These residues have previously been shown to reduce the affinity for the binding of polyamines to the SSAT protein, and these results indicate that the change in protein configuration brought about by this binding renders the protein resistant to proteasomal degradation. Mutations to alanines of residues arginine 7, cysteine 14, and lysine 141 also prevented the protection by BE-3-4-3, and these residues may be required for the formation of the protected conformation. The rapid degradation of SSAT required the carboxyl-terminal region of the protein, and the two terminal glutamic acid residues at positions 170 and 171 were found to be of critical importance. Truncation of the protein to remove these residues or the mutation of either of these acidic residues to glutamine completely abolished the rapid degradation of SSAT. The addition of two extra lysine residues at the carboxyl terminus or the conversion of the glutamic acids at positions 170 and 171 to lysines also prevented SSAT degradation by the proteasome. These results show the key role of the acidic residues at the carboxyl terminus of the protein in reacting with the proteasome. In contrast, mutation of lysine 166 to alanine, which extends the length of the acidic region in the carboxyl-terminal fragment of SSAT, actually increased the rate of degradation of SSAT without affecting its stabilization by BE-3-4-3. The binding of BE-3-4-3 or polyamines is therefore likely to change the configuration of the SSAT protein in a way that prevents the exposure of the carboxyl-terminal region of the ubiquitinated protein to the proteasome.

*S This research was supported in part by National Institutes of Health Grant GM-26290. 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.

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The abbreviations used are: SSAT, spermidine/spermine N$^1$-acetyltransferase; BE-3-4-3, N$^1$,N$^{12}$-bis(ethyl)spermine; ODC, ornithine decarboxylase; PAGE, polyacrylamide gel electrophoresis; ATP-S, adenosine 5'-O-(3-thiotriphosphate); calpain inhibitor I, N-acetyl-t-leucinyl-t-leucinal-t-norleucinal.

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an order of magnitude in the presence of BE-3-4-3 and other polyamine analogs. Experiments have been carried out in which the sensitivity of the SSAT protein and various mutants prepared by site-directed mutagenesis to degradation by proteases was used to investigate the binding of polyamine analogs to the protein and the subsequent conformational changes (22). There have been no reported studies on the mechanism of cellular degradation of SSAT or of the residues needed for the rapid turnover and for mediating the stabilization by polyamines.

In the present report we provide evidence that SSAT is a good substrate for degradation via the proteasomal/ubiquitin pathway (30–32), that this degradation is prevented by the binding of polyamines or BE-3-4-3 to the protein, and that interaction of either the proteasome requires the glutamic acid residues located at the carboxyl terminus of the protein. The similarity of the carboxyl end of the molecule to the PEST sequences known to be involved in the degradation of rapidly-turning over proteins including ODC is also discussed.

EXPERIMENTAL PROCEDURES

Materials—Oligodeoxynucleotides were synthesized in the Macromolecular Core facility, Hershey Medical Center, or were purchased from Life Technologies, Inc. pSAT9.3 was provided by Dr. J. M. Williams, University of Utah, Salt Lake City, UT. Full-length and 7.6-kDa fragments of human S5a proteasomal subunit were generously provided by Dr. Martin Rechsteiner, Department of Biochemistry, University of Utah, Salt Lake City, UT. Polyclonal anti-SSAT antibody was prepared as described previously (4).

Expression and Degradation of SSAT.—The plasmid pSAT9.3 containing the SSAT cDNA in Bluescript (19) and pGEM-ODC containing the ODC cDNA (33) were used to express the respective proteins from the T7 promoter of each vector. All mutations were introduced into the SSAT cDNA using pSAT9.3 as the template for site-directed mutagenesis as described previously (20, 22). The sequence of the entire coding region of all mutant cDNAs was checked to ensure the absence of frameshift mutations

Fig. 1. Amino acid sequence of human SSAT. Sites of mutations described in the text are indicated with bold characters. Residues that are essential for BE-3-4-3 induced stabilization are shown in bold, the glutamic acid residue found to be essential for rapid degradation is shown in bold with double underlining, and the lysine residue whose mutation was found to increase the rate of degradation is shown in bold with single underlining. The sites of cleavage of SSAT by trypsin or protease Glu-C are shown in italics.

Fig. 2. Effect of antizyme on the degradation of ODC and SSAT. The 35S-labeled wild type ODC (filled symbols) and wild type SSAT (open symbols) proteins were synthesized in the TNT-coupled transcription/translation system and used as substrates for degradation in the presence of an ATP regenerating system as described under “Experimental Procedures.” The proteins were incubated in the degradation assay at 37 °C in the presence of 0 (circles), 2 (squares), or 20 (triangles) ng of purified recombinant histidine-tagged antizyme. Aliquots of 50 μl were removed from the degradation assay at the times indicated, and proteolysis was determined by quantifying the intact labeled protein remaining following separation by SDS-PAGE and quantitated as described under “Experimental Procedures.”

by incubating 4-μl aliquots of the synthesis mix with crude rabbit reticulocyte lysate in a standard assay volume of 200 μl at 37 °C. Each degradation assay contained 40 μg Tris/HCl, pH 7.5, 5 mM MgCl2, 2 mM dithiothreitol, 0.5 mM ATP, 10 mM phosphocreatine, 0.05 mg/ml creatine phosphokinase, 0.1 mM cycloheximide, and 50 μl of reticulocyte lysate unless stated otherwise.

In experiments testing an energy requirement for SSAT degradation, ATP and the ATP regenerating system were replaced by 20 mM 2-deoxyglucose and 10 μM hexokinase. In experiments testing the effect of antizyme on the reaction, a polyhistidine-tagged preparation of antizyme was used. This was obtained by inserting the antizyme DNA containing a deletion of the thymidine present at position 205 (34) into the pQE-30 plasmid, expressing the protein in E. coli and purifying the resulting protein to homogeneity by immobilized metal affinity chromatography.

The rate of SSAT or ODC degradation was followed by removing 30-μl aliquots from the degradation assay at time intervals as shown in the legends to the figures. Aliquots were mixed with SDS sample buffer and boiled for 10 min prior to being resolved by SDS-PAGE. The rate of 35S-labeled protein degradation was determined by quantifying the fixed and dried gels using a Molecular Dynamics 425E-120 PhosphorImager and ImageQuant application software.

RESULTS

Degradation of SSAT and ODC in an ATP-dependent Reticulocyte Lysate System.—The degradation of SSAT was studied by using a reticulocyte lysate system shown previously to degrade ODC in a physiologically relevant manner (29, 33, 35). Labeled SSAT was synthesized using the TNT synthesis system with pSAT9.3, and aliquots were added to reticulocyte lysates. As shown in Fig. 2, SSAT was degraded very rapidly by these lysates, and the loss of the SSAT band occurred at a rate comparable to that of ODC. A maximal rate of ODC degradation required the addition of antizyme, which is known to be present in limited amounts in reticulocyte lysates (26), whereas antizyme had no effect on the rate of loss of SSAT.

The rapid degradation of the 35S-labeled SSAT required the presence of ATP and an ATP-regenerating system (results not shown). SSAT was stable during a 3-h incubation when ATP was omitted from the reaction and 2-deoxyglucose and hexokinase was included to deplete ATP pools present in the lysate. Substitution of ATP and the ATP-regenerating system with 2 μM of the nonhydrolyzable ATP analog, ATPγS, also resulted in a marked stabilization of SSAT with only a 30% loss in
shown, 30-35S-labeled SSAT in reticulocyte lysates led to the appearance of the 20-kDa band after 3 h of incubation. This small intensity of the 20-kDa band after 3 h of incubation. This small loss is likely to reflect the depletion of preexisting ATP pools in the lysate, as most of the degradation seen occurred early in the incubation.

Ubiquitin-mediated Degradation of SSAT—Incubation of the 35S-labeled SSAT in reticulocyte lysates led to the appearance of a ladder of higher molecular weight bands of labeled protein, which were readily visible in the films developed from the SDS-PAGE analysis (Figs. 3 and 4). The characteristic ladder was noticed to be more intense at earlier times, becoming fainter with extended incubation in the degradation reaction. To confirm that the higher molecular weight bands were associated with SSAT, aliquots taken at timed intervals from the degradation reaction were mixed with a polyclonal antibody to SSAT, and the immune complexes were precipitated upon addition of protein A and resolved by SDS-PAGE (Fig. 3). The higher molecular weight bands were precipitated by this antibody showing that the ladder does represent SSAT complexes. The size of the two lowest molecular weight complexes is consistent with the predicted size of mono- and di-ubiquitinated SSAT, suggesting that SSAT may be degraded via the ubiquitin-proteasome pathway.

As shown in Fig. 4, the rate of degradation of SSAT was reduced by the addition of calpain inhibitor I, which is one of a class of peptide aldehydes reported to be inhibitors of 20 S proteasomal function (36). This inhibitor also caused a persistence in the SSAT-ubiquitin bands. Since calpain inhibitor I is not absolutely specific for proteasomal proteases, the effect of a fragment of human S5a protein was also studied. The S5a protein (37) is a component of the 26 S proteasomal complex that has been shown to recognize and bind polyubiquitinated substrates (38, 39), thus targeting them for degradation by the 26 S proteasome. Rechsteiner and colleagues (40) showed that this protein acts as a potent and specific inhibitor of ubiquitin-dependent proteolysis. A fragment of this protein that contains the portion responsible for the inhibitory activity blocked SSAT degradation in a dose-dependent manner (Fig. 4). The characteristic ladder of ubiquitinated SSAT associated with SSAT degradation persisted longer in the presence of the S5a fragment (Fig. 4A). The addition of 1.5 mg/ml S5a fragment led to about 58% of the 20-kDa band corresponding to SSAT remaining after a 3-h incubation at 37 °C (Fig. 4B).

Effect of Polyamines and BE-3-4-3 on SSAT Degradation—The degradation of 35S-labeled SSAT became progressively slower with increasing concentrations of spermidine and spermine added to the degradation assay (Fig. 5). Spermine was more effective than spermidine in preventing SSAT degradation with 73% compared with 36% of the 20-kDa band remaining after a 2-h incubation in the presence of 1 mM spermine or spermidine, respectively. BE-3-4-3 was more potent than either of the natural polyamines, giving maximal protection of SSAT from degradation in the reticulocyte lysate system at 100 μM concentrations (Fig. 6). It is known that either BE-3-4-3 (22) or much higher albeit physiological levels of natural polyamines2 produce a conformational change in SSAT structure, and the results shown in Figs. 6 and 7 therefore indicate that this conformational change renders SSAT resistant to degradation by the ubiquitin-dependent system present in the reticulocyte lysates. Addition of BE-3-4-3 did not prevent the ubiquitination of the SSAT protein, since the higher molecular weight bands were still present when either wild type or the K166A mutant SSAT described below were incubated with the lysates supplemented with BE-3-4-3 (re-
Effect of Mutations in SSAT on Proteasomal Degradation—To investigate the structural features of SSAT responsible for the rapid proteasomal degradation and for the prevention of this degradation by BE-3-4-3, a series of alterations to the SSAT sequence (Fig. 1) were made by site-directed mutagenesis, and the mutant proteins were incubated for varying periods of up to 3 h in the presence or absence of BE-3-4-3. Results for the amount of degradation that occurred in 1 h, which were representative of the results of the entire time courses, are shown in Fig. 7. The gels showing results for selected mutants of particular interest are shown in Fig. 6.

The rapid degradation of SSAT in the absence of BE-3-4-3 requires the carboxyl end of the molecule, since mutants E170Stop, A168Stop, and M167Stop were not rapidly degraded (Fig. 7A). The critical importance of the carboxyl side chain of the terminal two glutamic acid residues is shown by the results with point mutations where either was altered to glutamine. Both of these mutants (E170Q and E171Q) were stable (Figs. 6A and 7A). Replacing these two acidic residues with basic lysines (mutant E170K/E171K) or adding two lysines as additional residues to the end of the SSAT sequence (mutant +172K/173K) also rendered the SSAT stable (Fig. 7A). The SSAT mutants rendered stable by mutation or truncation of the carboxyl domain were still subject to ubiquitination since the higher molecular weight bands similar to those seen in Figs. 3 and 4 were still seen with mutants E170Stop, E171Q, E170Q, and E170K/E171K (results not shown). Only changes in the carboxyl end of the protein caused the SSAT to become resistant to proteasomal degradation. None of the other point mutations studied (which encompass residues throughout the molecule) prevented the rapid degradation of the SSAT protein (Fig. 7). The triple mutant K141S/R142A/R143S did prevent rapid degradation of SSAT (Fig. 6B). This mutation removes the site of tryptic digestion (22) of pure SSAT but may distort the structure of the protein since this mutation also renders the protein completely inactive.

Mutant K166A was degraded even more rapidly than the wild type protein. This effect is seen in the data shown in Fig. 7A but is underestimated because even the control protein is substantially degraded in the 1-h time period. Fig. 6A, in which the entire time course is shown, indicates clearly that this mutation substantially increases the rate of degradation of the SSAT.

However, this K166A mutation did not prevent the ability of BE-3-4-3 to stabilize the SSAT protein. Several mutations did reduce or totally abolish the protective effect of BE-3-4-3. These included mutants M167A, R155A, E152K (and E152Q), H126A, C122A, and R19A (Figs. 6B and 7). All of these mutations have been shown to reduce the ability of SSAT to bind polyamines or polyamine analogs (20, 22). Mutations R7A, C14A, and K141A also prevented the stabilization by BE-3-4-3 (Fig. 7, B and C). In addition to providing information on the residues responsible for stabilization by BE-3-4-3, these observations rule out the possibility that BE-3-4-3 inhibits the degradation system directly.

Discussion

Evaluation of the properties of protein mutants generated by site directed mutagenesis is sometimes subject to question because of the possibility that the protein structure is distorted as a result of the mutation. However, this is unlikely to account for our results since the key mutations have previously been tested and shown to produce little or no alteration in SSAT enzymatic activity or in the characteristic pattern of SSAT sensitivity to proteases (20, 22). Previous studies have shown
the region covering amino acids 166–171, findings that SSAT degradation by the ubiquitin-proteasomal system is greatly reduced by BE-3-4-3 is in agreement with these observations. It appears likely that this protection is mediated by an alteration in configuration in the SSAT protein brought about by binding the polyamine analog. Most of the mutations that were found in the experiments illustrated by Figs. 6 and 7 to abolish the stabilization of SSAT by BE-3-4-3 have also been shown to abolish the protection of the SSAT protein from protease cleavage and to increase the \( K_m \) for spermidine in the acetylation reaction. These include the mutants M167A, R155A, E152K or E152Q, H126A, C122A, and R19A (20, 22). Therefore, it is likely that the inability of BE-3-4-3 to stabilize these mutant SSATs in the proteasomal degradation system is due to a lack of binding of the polyamine analog. Although these mutations do have reduced levels of SSAT activity, it is unlikely that they cause major alterations in the structure of SSAT since (a) the major part of the reduction in activity is due to a change in the \( K_m \) for polyamines, and (b) the mutant E152K forms enzymatically active heterodimers when co-expressed with mutant R101A which forms part of the acetyl-CoA binding site (20).

A simple model that would account for our findings would be that SSAT degradation is brought about by ubiquitination of the protein that causes it to be bound to the proteasomal structure. Degradation then requires the interaction of the proteosome with the carboxyl end of the SSAT, and this interaction requires the carboxyl side chains of the glutamic acid residues 170 and 171. The binding of BE-3-4-3 or polyamines alters the configuration of the protein so that this carboxyl end is not exposed and the degradation is therefore prevented. Several peptide motif sequences have been proposed to target intracellular proteins for rapid destruction (41). The terminal -MATEE motif of the SSAT appears to provide another example of such a sequence. The most widespread motif that has been hypothesized to mark proteins for rapid turnover is the PEST sequence, which is defined as a region of \( >12 \) amino acids that contains proline, serine/threonine, and glutamic acid/aspartic acid in the absence of basic residues (41, 42). The PEST hypothesis has received much support from the almost ubiquitous presence of such PEST sequences in rapidly turning over proteins, although it has not yet been determined how the sequences act. SSAT (Fig. 1) does not contain an obvious PEST sequence. The terminal -MATEE motif, which is identified in the current study as a critical region for SSAT turnover, is eliminated from consideration by the absence of a proline residue, but the other components of the PEST motif, acidic residues and serine or threonine, are present in the carboxyl-terminal sequence, and the carboxyl-terminal location may permit this sequence to be exposed for interaction with the proteolytic machinery without the need for a proline. Furthermore, positively charged residues are not compatible with PEST sequences, and the mutation to alanine of lysine 166, which increases the liability of SSAT, extends the length of the acidic carboxyl-terminal sequence. It is therefore possible that -MATEE acts as a “pseudo-PEST” sequence.

Although both enzymes lead to an increase in putrescine in the cell, ODC and SSAT have opposing roles in polyamine synthesis, with ODC tending to increase and SSAT to decrease polyamine levels (24). Both enzymes are regulated at the level of protein stability by polyamines but in opposite directions. ODC content is reduced by polyamines via the antizyme-mediated enhancement of degradation, whereas SSAT is increased by polyamines via the prevention of degradation. In both cases, the carboxyl end of the protein is a critical region for interaction with the proteasome. Removal of from 5 to 37 residues from the carboxyl terminus of ODC (43, 44), or the point mutation of cysteine 441, which is contained within this region.
renders ODC stable without affecting its enzymatic activity. Attaching the same 37 residues of ODC to the terminus of dihydrofolate reductase rendered this protein subject to rapid degradation (46). It will be of interest to determine whether the carboxyl-terminal domain of SSAT is also able to impart rapid turnover to other proteins.

Acknowledgments—We thank Dr. M. Rechsteiner for the gift of protein SSa fragment and D. Feith for the antizyme preparation.

REFERENCES
1. Casero, R. A., and Pegg, A. E. (1993) FASEB J. 7, 653–661
2. Seiler, N. (1987) Can. J. Physiol. Pharmacol. 65, 2024–2035
3. Wallace, H. M., and Quick, D. M. (1994) Biochem. Soc. Trans. 22, 870–875
4. Casero, J., R. A., Gabrielson, E. W., and Pegg, A. E. (1994) Cancer Res. 54, 3955–3958
5. Persson, L., and Pegg, A. E. (1984) J. Biol. Chem. 259, 12364–12367
6. Pegg, A. E. (1986) Biochem. J. 234, 249–262
7. Casero, R. A., Celano, P., Ervin, S. J., Porter, C. W., Bergeron, R. J., and Libby, P. (1989) Cancer Res. 49, 3829–3833
8. Casero, R. A., Mank, A. R., Xiao, L., Smith, J., Bergeron, R. J., and Celano, P. (1992) Cancer Res. 52, 5359–5363
9. Davidson, N. E., Mank, A. R., Prestigiacomo, L. J., Bergeron, R. J., and Casero, R. A. (1993) Cancer Res. 53, 2071–2075
10. Porter, C. W., Ganis, B., Libby, P. R., and Bergeron, R. J. (1991) Cancer Res. 51, 3715–3720
11. Saab, N. H., E., W. E., Bieszk, N. C., Preuss, C. V., Mank, A. R., Casero, R. A., and Woster, P. M. (1993) J. Med. Chem. 36, 229–262
12. Libby, P. R., Henderson, M., Bergeron, R. J., and Porter, C. W. (1989) Cancer Res. 49, 6226–6231
13. Fogel-Petricov, M., Shappell, N. W., Bergeron, R. J., and Porter, C. W. (1993) J. Biol. Chem. 268, 19118–19125
14. Fogel-Petricov, M., Vujicic, S., Brown, P. J., Haddox, M. K., and Porter, C. W. (1996) Biochemistry 35, 14436–14444
15. Perry, L., Balan Foue, R., and Pegg, A. E. (1995) Biochem. J. 305, 451–458
16. Matsui, I., and Pegg, A. E. (1981) Biochem. Biophys. Acta 675, 373–378
17. Erwin, B. G., and Pegg, A. E. (1986) Biochem. J. 230, 581–587
18. Libby, P. R., Bergeron, R. J., and Porter, C. W. (1989) Biochem. Pharmacol. 38, 1435–1442
19. Casero, R. A., Jr., Celano, P., Ervin, S. A., Applegren, N. B., Wiest, L., and Pegg, A. E. (1991) J. Biol. Chem. 266, 810–814
20. Coleman, C. S., Huang, H., and Pegg, A. E. (1996) Biochem. J. 316, 697–701
21. Lu, L., Berkey, K. A., and Casero, R. A., Jr. (1996) J. Biol. Chem. 271, 18920–18924
22. Coleman, C. S., Huang, H., and Pegg, A. E. (1995) Biochemistry 34, 13423–13430
23. Tabor, C. W., and Tabor, H. (1984) Annu. Rev. Biochem. 53, 749–790
24. Pegg, A. E. (1988) Cancer Res. 48, 759–774
25. Hayashi, S., Murakami, Y., and Matsufuji, S. (1996) Trends Biochem. Sci. 21, 27–30
26. Hayashi, S., and Murakami, Y. (1995) Biochem. J. 306, 1–10
27. Li, X., and Coffino, P. (1994) Mol. Cell. Biol. 14, 87–92
28. Elias, S., Bercovich, B., Kahana, C., Coffino, P., Fischer, M., Hilt, W., D. H., and Ciechanover, A. (1995) Eur. J. Biochem. 229, 276–283
29. Bercovich, Z., and Kahana, C. (1993) Eur. J. Biochem. 213, 205–210
30. Ciechanover, A. (1994) Cell 79, 13–21
31. Coux, O., Tanaka, K., and Goldberg, A. L. (1996) Annu. Rev. Biochem. 65, 801–847
32. Hilt, W., and Wolf, D. H. (1996) Trends Biochem. Sci. 21, 96–101
33. Libby, P. R., Bergeron, R. J., and Porter, C. W. (1989) Cancer Res. 51, 3715–3720
34. Pegg, A. E. (1986) Biochem. J. 234, 249–262
35. Saab, N. H., E., W. E., Bieszk, N. C., Preuss, C. V., Mank, A. R., Casero, R. A., and Woster, P. M. (1993) J. Med. Chem. 36, 229–262
36. Libby, P. R., Henderson, M., Bergeron, R. J., and Porter, C. W. (1989) Cancer Res. 49, 6226–6231
37. Fogel-Petricov, M., Shappell, N. W., Bergeron, R. J., and Porter, C. W. (1993) J. Biol. Chem. 268, 19118–19125
38. Fogel-Petricov, M., Vujicic, S., Brown, P. J., Haddox, M. K., and Porter, C. W. (1996) Biochemistry 35, 14436–14444
39. Perry, L., Balan Foue, R., and Pegg, A. E. (1995) Biochem. J. 305, 451–458
40. Matsui, I., and Pegg, A. E. (1981) Biochem. Biophys. Acta 675, 373–378
41. Erwin, B. G., and Pegg, A. E. (1986) Biochem. J. 230, 581–587
42. Libby, P. R., Bergeron, R. J., and Porter, C. W. (1989) Biochem. Pharmacol. 38, 1435–1442
43. Casero, R. A., Jr., Celano, P., Ervin, S. A., Applegren, N. B., Wiest, L., and Pegg, A. E. (1991) J. Biol. Chem. 266, 810–814
44. Coleman, C. S., Huang, H., and Pegg, A. E. (1996) Biochem. J. 316, 697–701
45. Loetscher, P., Pratt, G., and Rechsteiner, M. (1991) J. Biol. Chem. 266, 11215–11220
46. Loetscher, P., Pratt, G., and Rechsteiner, M. (1991) J. Biol. Chem. 266, 11215–11220
47. Loetscher, P., Pratt, G., and Rechsteiner, M. (1991) J. Biol. Chem. 266, 11215–11220
