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IN VITRO ADP-RIBOSYLATION OF AMP-DEAMINASE AND ITS EFFECT ON THE PROTECTION OF THE ADP-BINDING DOMAIN AGAINST PROTEOLYSIS[1]

(ADP-ribose / AMP-deaminase / regulation)

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We investigated the ADP-ribosylation of AMP-deaminase from rat and rabbit skeletal muscle by guanidino compound-specific ADP-ribosyltransferase from hen liver nuclei and its effect on the enzyme activity. Rat AMP-deaminase was ADP-ribosylated when the enzyme was incubated with ADP-ribosyltransferase and [adenylate-^{32}P] NAD. Preincubation of rabbit AMP-deaminase alone resulted in a loss of ADP-dependent, but not basal, enzyme activity. If, however, the enzyme was subjected to the ADP-ribosylation system containing unlabeled NAD and ADP-ribosyltransferase, the enzyme retained the property to be activated by ADP. Electrophoretic analyses of the enzyme preparation incubated with or without the ADP-ribosylation system and subsequently further incubation with trypsin showed that the ADP-ribosylation of the enzyme protects the enzyme from proteolysis concomitant with the retention of ADP-dependent activation of the enzyme.

Eukaryote guanidino compound-specific ADP-ribosyltransferase activity was first observed in turkey erhtyrocytes, and the enzyme was purified and characterized (1,2). Subsequently, this enzyme activity was detected in human erythrocytes (3), rat liver

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3. Abbreviation: SDS, sodium dodesyl sulphate.

(4) and thyroid (5), hen liver nuclei (6) and sarcoplasmic reticulum from mammalian skeletal muscle (7). Although the precise role of the enzyme remained to be determined, ADP-ribosylation-induced changes in the activities and/or regulatory properties of certain enzymes have been reported. In 1984, Moss et al. (8) demonstrated that the ADP-ribosylation of glutamine synthetase purified from bovine brain and hen heart resulted in their complete inactivation. Recently, it was demonstrated that ADP-ribosylation of acetyl CoA carboxylase from rat liver by cholera toxin led to an inhibition of the enzyme owing to a decrease in Vmax and an increase in the apparent Ka for an allosteric activator citrate (9). Using ADP-ribosylated phosphorylase kinase, the functional relationship between ADP-ribosylation and cAMP-dependent phosphorylation has been demonstrated (10). ADP-ribosylation inhibited phosphorylation of the phosphorylase kinase by a cAMP-dependent protein kinase. The same was true in the case of bovine thymus H1 histone phosphorylation (11). According to the report by Ranieri-Raggi et al. (12), phosphorylation and dephosphorylation of AMP-deaminase from rabbit skeletal muscle are directly related to the changes in the enzyme activity. All these data provide the background for our studies of ADP-ribosylation of AMP-deaminase from mammalian skeletal muscle.

Here, we report the evidence for in vitro ADP-ribosylation of AMP-deaminase from rat and rabbit skeletal muscle by hen liver nuclear ADP-ribosyltransferase and we report also that ADP-ribosylation of the enzyme protects the ADP-binding domain against endogenous or exogenous proteolysis, which domain functions for allosteric activation of the enzyme.

MATERIALS AND METHODS

Materials

Albino male rabbits and white male Wistar rats were maintained on an Oriental Yeast solid diet and tap water ad libitum. [adenylate-^{32}P] NAD(960 Ci/mmol) was obtained from New England Nuclear. NAD was obtained from Sigma Chemical Co. All other reagents were purchased from Miyata Chemicals Co. Ltd, Shimane, and were used without further purification.

Methods

Preparation of enzymes --- AMP-deaminases from rabbit and rat skeletal muscle were purified by the method of Smiley et al. (13). ADP-ribosyltransferase was purified from hen liver nuclei by a modification of the method reported previously from our laboratory (6). Details of the modified purification method will be described elsewhere. In brief, the purified nuclei were suspended in 0.35 M NaCl, homogenized, and the enzyme was purified by subsequent chromatography, successively on hydroxyapatite, phenyl-Sepharose and Cm-cellulose. The specific activity of the ADP-ribosyltransferase in the final preparation, determined in the presence of 2 mg/ml of casein as acceptor, under the conditions described (6), was 50 μmol/mg/h.

Assays --- The basal AMP-deaminase activity was measured spectrophotometrically at 285 nm at 25°C using a Hitachi spectrophotometer model 100-60. The reaction mixture for the assay contained 50 ng of the enzyme protein, 2.5 mM AMP, 5 mM dithiothreitol and 50 mM imidazole-HCl buffer, pH 6.5. in a total volume of 2.0 ml. For assay of the ADP-dependent AMP-deaminase activity, the substrate and ADP were added at concentrations of 0.25 mM and 50 μM, respectively. The other conditions were the same as the assay for the basal activity.

ADP-ribosylation of AMP-deaminase --- Twenty to twenty five μg of the enzyme preparation were incubated in 50 μl of reaction mixture containing 50 mM imidazole-HCl buffer, pH 6.5, 5 mM dithiothreitol, 0.5 mM [adenylate-^{32}P] NAD (200,000-400,000 cpm), and 50 ng of ADP-ribosyltransferase for indicated time at 25°C. The reaction was stopped by adding 10% trichloroacetic acid. Radioactivity of the acid-insoluble fraction was measured by liquid scintillation counting using a Packard Liquid Scintillation Spectrometer.

Proteolysis of AMP deaminase --- Limited proteolysis of AMP deaminase with trypsin was performed as described by the method of Ranieri-Raggi et al. (14).

Gel-electrophoresis --- SDS-polyacrylamide gel electrophoresis was carried out on 10% polyacrylamide gel containing 0.1% SDS (16). Phosphorylase b (94 k), bovine serum

albumin (67 k), ovalbumin (43 k) and carbonic anhydrase (30 k) were used as marker proteins. Before applying to the gel, the samples were denatured in a medium containing 10 m M Tris-HCl buffer (pH 6.8), 2.5% SDS, 5% mercaptoethanol and 10% sucrose. After electrophoresis, the gels were stained with 0.2% Coomassie brilliant blue. Gels were scanned using a Zeinen Soft Lazer Scanning Densitometer (Biomed Instruments, USA). To obtain autoradiographs, the dry gels were exposed to Kodak RXO-G film for 3-7 days at -40°C.

RESULTS

Evidence for ADP-ribosylation of AMP-deaminase --- To determine whether or not AMP-deaminase serves as an acceptor for ADP-ribosylation reaction, the purifed AMP-deaminase from rat skeletal muscle was incubated with ADP-ribosyltransferase purified from hen liver nuclei and [^{32}P] NAD for 4 h at 25°C. After incubation, the acid-insoluble fraction of the reaction mixture was subjected to 10% polyacrylamide gel electrophoresis. As shown in Fig. 1, radioactive band was observed only in the area corresponding with AMP-deaminase (70 k). Under the incubation conditions similar to the Fig. 1 experiment, the amounts of ADP-ribose incorporated into the acid-insoluble fraction of the reaction mixture containing AMP-deaminase from rat and rabbit skeletal muscle were 0.05 mol/mol subunit/h and 0.62 mol/mol subunit/h, respectively. Accordingly, AMP-deaminase purified from rabbit skeletal muscle was used for the following experiments.

pH optimum --- Effect of pH on the ADP-ribosylation of rabbit AMP-deaminase by the ADP-ribosyltransferase was determined over a pH range of 7 units with imidazole-HCl buffer (pH 6.5 to 7.5), Tris-HCl buffer (pH 8.0 to 9.0) and glycine-HCl buffer (pH 9.5 to 10.0), at a concentration of 50 mM. The optimum pH was observed at 9.0, as reported with other acceptors for ADP-ribosylation (6, 8, 9). However, we noticed that an alkaline treatment of the AMP-deaminase brought about an inactivation of the enzyme (data not shown). Thus, we used imidazole buffer, pH 6.5, for ADP-ribosylation of AMP-deaminase.

Time course of ADP-ribosylation --- The amount of

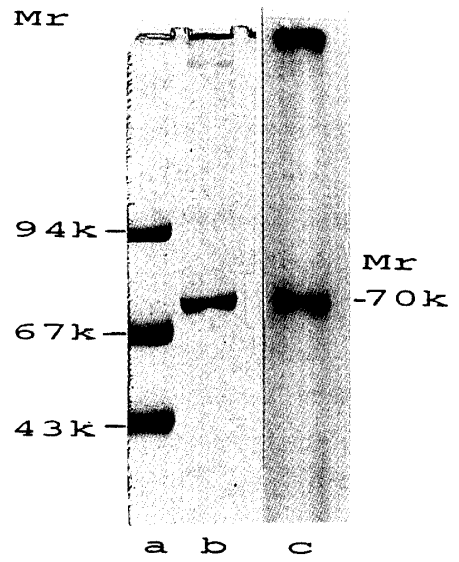

Fig.1. ADP-ribosylation of AMP-deaminase purified from rat skeletal muscle. Twenty five µg of AMP-deaminase were incubated in a total volume of 50 µl of reaction mixture containing 0.5 mM [adenylate-^{32}P] NAD (400,000 cpm), 50 ng of ADP-ribosyltransferase, 5 mM dithiothreitol and 50 mM imidazole-HCl buffer, pH 6.5 for 4 h at 25°C. The reaction was stopped by adding 10% trichloroacetic acid. Radiolabeling of the acid-insoluble fraction (17 µg protein) was analyzed by SDS-polyacrylamide gel electrophoresis, as described in "Methods". The Commassie brilliant blue-staining pattern (b), an autoradiogram of the same gel (c) and molecular weight markers (a) were shown.

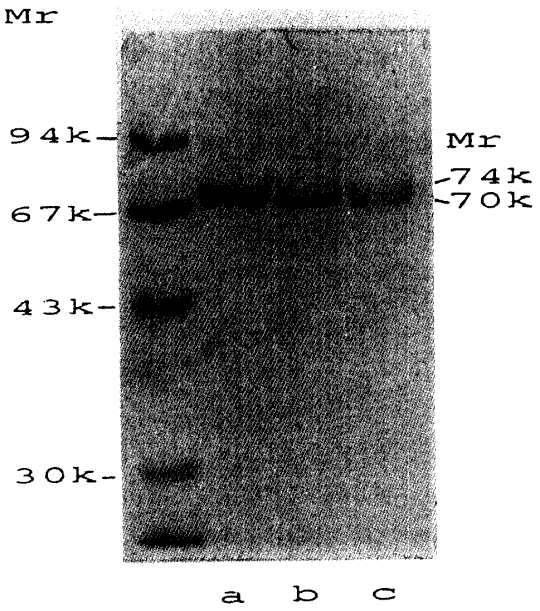

Fig. 2. SDS-polyacrylamide gel electrophoresis of rabbit AMP-deaminase. Fifty µg of the purified AMP-deaminase from rabbit skeletal muscle were incubated at 25°C for 4 h without or with ADP-ribosylation system containing unlabeled 0.5 mM NAD and 50 ng of ADP-ribosyltransferase in 50 µl of 50 mM imidazole-HCl buffer, pH 6.5 and 5 mM dithiothreitol. The reaction was terminated by adding 10% trichloroacetic acid. The acid-insoluble fraction (15 µg of protein) was analyzed by 10 % polyacrylamide gel electrophoresis in 0.1 % SDS. After electrophoresis the protein band was visualized by staining with Coomassie brilliant blue. Non-incubated (a), incubated without ADP-ribosylation system (b) and incubated with ADP-ribosylation system (c). The scale on the left shows the positions of the Mr markers run simultaneously with each slab.

ADP-ribosylation as a function of incubation time was tested at pH 6.5. The ADP-ribosylation showed a linearity with time over 6 h, when 50 ng of ADP-ribosyltransferase and 25 µg of the AMP-deaminase as acceptor were added to 50 µl of the reaction mixture. Furthermore, the amounts of ADP-ribosyl AMP-deaminase were in direct proportion to the amount of ADP-ribosyltransferase added, up to 200 ng (data not shown).

Effect of ADP-ribosylation on AMP-deaminase activity --- To see the effect of ADP-ribosylation on AMP-deaminase activity, AMP-deaminase purified from rabbit skeletal muscle was preincubated with NAD, ADP-ribosyltransferase or both at pH 6.5 and an aliquot of each reaction mixture was directly subjected to AMP-deaminase assay, in which 2.5 mM AMP was contained as substrate. For controls, AMP-deaminase was assayed without preincubation, but in the presence of the compounds listed in Table I. As shown in the table, addition of these compounds during preincubation increased the enzyme activity to some extent. Concentrations of bovine serum albumin and propylene glycol were adjusted to those of ADP-ribosyltransferase used here and of the glycol present in the ADP-ribosyltransferase preparation, respectively. The preincubation of AMP-deaminase without addition or with NAD alone drastically decreased the enzyme activity, while those with ADP-ribosyltransferase alone or plus NAD evidently increased it. Furthermore, addition of bovine serum albumin plus propylene glycol or bovine serum albumin plus NAD was also effective for protection against preincubation-induced suppression of the enzyme activity. Thus, the retention of enzyme activity during preincubation can not be attributed to the ADP-ribosylation of AMP-deaminase. Concerning the preincubation-induced suppression of the enzyme activity, it is possible to speculate that a certain amount of endogenous/intrinsic protease(s) present in the AMP-deaminase preparation may cleave the deaminase leading to an inactivation of the enzyme, while in the presence of ADP-ribosyltransferase or bovine serum albumin the rate of AMP-deaminase inactivation can be lessened, because the protease(s) may attack such proteins, too. To see this point further, AMP-deaminase purified from rabbit skeletal muscle was incubated with and without the ADP-ribosylation system and subjected to gel electrophoresis followed by staining. For control, the enzyme preparation not incubated was analyzed at the same time. As shown in Fig. 2, the control preparation revealed two bands corresponding to 70 and 74 k subunits as reported by Rainieri-Raggi and Raggi (14, 16). The densitometric determination of the gels showed that the ratio of 70 k to 74 k in the control was to be 1.23, while those in the preparations incubated with and without the ADP-ribosylation system were 2.25 and 3.03, respectively. This means that the ADP-ribosylation may prevent proteolysis of the larger subunit

(74 k) to the smaller one (70 k). From the results shown in Fig. 2 and Table I, we presume that the limited proteolysis of the 74 k subunit does not influence the basal AMP-deaminase activity.

To further investigate the effect of ADP-ribosylation on proteolysis of the AMP-deaminase, the enzyme preincubated with or without the ADP-ribosylation system was further incubated with trypsin, and the respective material was analyzed by gel electrophoresis followed by staining (Fig. 3). As expected, ADP-ribosylation clearly suppressed the conversion of 74 k subunit to 70 k; in the absence of the ADP-ribosylation system, the density of the band corresponding to 74 k largely decreased and that of the other band corresponding to 70 k increased, while such conversion was not seen in the presence of the ADP-ribosylation system (Fig. 3). These results indicate that ADP-ribosylation is effective for protection of the enzyme against exogenous proteolysis, and the following question was raised: does ADP-ribosylation influence the functional modification of the enzyme?

In an attempt at resolution, rabbit AMP-deaminase was treated with or without ADP-ribosylation system followed by treatment with trypsin and then the respective preparation was subjected to the enzyme assay with 0.25 mM AMP as substrate in

Table I. EFFECT OF PREINCUBATION OF AMP-DEAMINASE WITH AND WITHOUT ADP-RIBOSYLATION SYSTEM ON THE ENZYME ACTIVITY

Addition	Activity (Δ OD_{285}/min/100 µg)	
	Non-preincubated	Preincubated
None	1.50	0.90
NAD (0.5 mM)	1.73	0.95
ADP-ribosyltransferase (50 ng)	1.73	2.20
ADP-ribosyltransferase (50 ng) plus NAD (0.5 mM)	1.73	2.25
Bovine serum albumin (50 ng) plus propylene glycol (2 µg)	1.83	2.03
Bovine serum albumin (50 ng) plus NAD (0.5 mM)	1.88	1.93

Twenty µg of AMP-deaminase were preincubated at 25°C for 4 h with compounds listed in the table, in 50 µl of 50 mM imidazole-HCl buffer, pH 6.5 containing 5 mM dithiothreitol. After preincubation, an aliquot (4 µg of AMP-deaminase) of the respective mixture was directly subjected to an AMP-deaminase assay system (2.0 ml) containing 2.5 mM AMP as substrate and 50 mM imidazole-HCl buffer, pH 6.5. The other conditions were as described in " Methods".

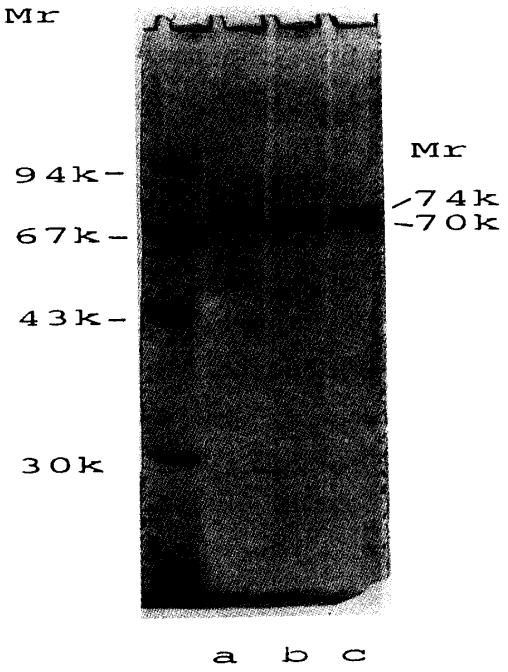

Fig. 3. SDS-polyacrylamide gel electrophoresis of rabbit AMP-deaminase incubated with ADP-ribosylation system followed by trypsin treatment. Fifty µg of AMP-deaminase were preincubated with or without ADP-ribosylation system as described in the legend to Fig. 2. After incubation for 2 h at 25°C, an aliquot (30 µg of protein) of the each mixture was further incubated with trypsin (50 µg/ml) for 30 min at 25°C. The reaction was terminated by adding 10% trichloroacetic acid. For control, AMP-deaminase preincubated without ADP-ribosylation system was further incubated with trypsin. After electrophoresis the protein band was visualized by staining with Coomassie brilliant blue. Non-incubated (a), preincubated with ADP-ribosyltransferase and then treated with trypsin (b) and preincubated without ADP-ribosylation system and treated with trypsin (c). The scale on the left shows the positions of the Mr markers run simultaneously with each slab.

Table II. EFFECT OF ADP-RIBOSYLATION OF AMP-DEAMINASE BEFORE TRYPSIN TREATMENT ON THE ADP-DEPENDENT ENZYME ACTIVITY

Conditions	Activity (Δ OD_{285}/min/100 µg)	
	- ADP	+ ADP
Not preincubated	0.60	2.38
Preincubated with		
None	0.73	0.83
ADP-ribosyltransferase (50 ng) plus NAD (0.5 mM)	1.10	3.63
Bovine serum albumin (50 ng), propylene glycol (2 µg) plus NAD (0.5 mM)	1.38	1.50
ADP-ribosyltransferase (50 ng), NAD (0.5 mM) plus nicotinamide (50 mM)	1.38	1.06

Twenty µg of AMP-deaminase were preincubated at 25°C for 2 h with compounds listed in the table, in 50 µl of 50 mM imidazole-HCl buffer, pH 6.5 containing 5 mM dithiothreitol. The mixture was further treated with 50 µg/ml of trypsin for 30 min at 25°C and the proteolysis was stopped by addition of phenylmethanesulfonyl fluoride at concentrations of 10 mM. Aliquot (0.8 µg of AMP-deaminase) of the each mixture was directly subjected to AMP-deaminase assay system (2.0 ml) containing 0.25 mM AMP as substrate and 50 mM imidazole buffer, pH 6.5 and 50 µM ADP or not.

the presence and absence of allosteric effector ADP. As can be seen in Table II, the enzyme preparation preincubated with the ADP-ribosylation system before trypsin treatment, retained the ADP-dependent activation of AMP-deaminase. This was further confirmed by the addition of nicotinamide, a potent inhibitor of ADP-ribosylation reaction, into the ADP-ribosylation system; ADP-dependent activation of AMP-deaminase was not detected, nor was preincubation without the ADP-ribosylation system. The presence of bovine serum albumin, propylene glycol plus NAD during preincubation before trypsin treatment retained ADP-induced AMP-deaminase activity to some extent.

We also tested to see whether or not ADP-ribosylation influences the effects of allosteric inhibitors such as K^+ and GTP on AMP-deaminase activity, but this was not the case (data not shown).

DISCUSSION

We demonstrated that the purified AMP-deaminases from rat and rabbit skeletal muscle are ADP-ribosylated by hen liver nuclear ADP-ribosyltransferase. The data reported here indicate that the ADP-ribosylation of one arginine residue of AMP-deaminase brought about suppression of the limiting proteolysis of the enzyme by either endogenous or exogenous protease.

Concerning the construction of AMP-deaminase, Ranieri-Raggi and Raggi (14, 16) reported that the rabbit skeletal muscle enzyme consists of two subunits having molecular weights of 79 and 73 k. They also demonstrated that the limited proteolysis of the enzyme by trypsin caused a disappearence of the larger subunit and the appearance of one band corresponding to the protein with molecular weight of 72±2 k. These treatments also led to a loss of enzyme sensitivity to ADP and brought about unusual hyperbolic kinetics at low concentrations of potassium.

Here, we report the trypsin-directed conversion of the larger subunit of AMP-deaminase to the smaller one concomitant with desensitization of the enzyme to ADP and ADP-ribosylation-induced protection of ADP-binding domain against the limited proteolysis by endogenous or exogenous protease. Concerning the structure of AMP-deaminase, Coffee and Kofke demonstrated that the tryptic finger printing of AMP-deaminase

from rabbit skeletal muscle has 22 arginine-containing peptides in each subunit of the enzyme (17). ADP-ribosyltransferase used in the present experiment is guanidino compound-specific enzyme. Thus, we presume that ADP-ribosylation of the AMP-deaminase may occur on the specific arginine of the enzyme and that ADP-ribose-acceptable arginine may be sequenced closely to the ADP-binding site. Alternatively, ADP-ribosylation of the enzyme may change the conformation of the enzyme to one less accessible to the protease. Riquelme et al. (18) have demonstrated that ADP-ribosylation of H1 histone resists a proteolysis.

Recently, guanidino compound-specific ADP-ribosyltransferase activity was observed in mammalian skeletal muscle (7). On the basis of these findings together with our data described here, we presume that AMP-deaminase from rabbit skeletal muscle may serve as an acceptor for ADP-ribosylation by guanidino compound-specific ADP-ribosyltransferase in vivo and that this modification allows the stabilization of the ADP-binding domain which is capable of serving as an allosteric regulation of the enzyme. Further studies are required to support our hypothesis that ADP-ribosylation may play a role as a significant regulator of the AMP-deamination reaction in vivo.

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REFERENCES

1. Moss, J. and Vaughan, M. (1978) Isolation of an avian erythrocyte protein possesing ADP-ribosyltransferase activity and capable of activating adenylate cyclase. Proc. Natl. Acad. Aci. USA, 75, 3621-3624
2. Moss, J., Stanley, S. J. and Watkins, P. A. (1980) Isolation and properties of an NAD- and guanidine-dependent ADP-ribosyltransferase from turkey erythrocytes. J. Biol. Chem. 255,5838-5840
3. Moss, J. and Stanley, S. J. (1981) Histone-dependent and histone-independent forms of an ADP-ribosyltransferase from human and turkey erythrocytes. Proc. Natl. Acad. Sci. USA, 78, 4809-4812

4. Moss, J. and Stanley, S. J. (1981) Amino acid-specific ADP-ribosylation. Identification of an arginine-dependent ADP-ribosyltransferase in rat liver. J. Biol. Chem., 256, 7830-7833
5. De Wolf, M. J. S., Vitti, P., Ambesi-Impiobato, F. S. and Kohn, L. D. (1981) Thyroid membrane ADP-ribosyltransferase activity. Stimulation of thyrotropin and activity in functioning and nonfunctioning rat throid cells in culture. J. Biol. Chem., 256, 12287-12295
6. Tanigawa, Y., Tsuchiya, M., Imai, Y. and Shimoyama, M. (1984) ADP-ribosyltransferase from hen liver nuclei. Purification and characterization. J. Biol. Chem., 259, 2022-2029
7. Soman, G., Mickelson, J. R., Lovis, C. F. and Graves, D. J. (1984) NAD: guanidino group specific mono ADP-ribosyltransferase activity in skeletal muscle. Biochem. Biophys. Res. Commun., 120, 973-980
8. Moss, J., Watkins, P. A., Stanley, S. J., Purnell, M. R. and Kidwell, R.(1984) Inactivation of glutamine synthetase by NAD:arginine ADP-ribosyltransferase. J. Biol. Chem., 259, 5100-5104
9. Witters, L.A., Mc Dermott, M. (1986) Regulation of acetyl-CoA carboxylase by ADP-ribosylation. Biochemistry, 25, 7216-7220
10. Tsuchiya, M., Tanigawa, Y., Ushiroyama, T., Matsuura, R. and Shimoyama, M. (1985) ADP-ribosylation of phosphorylase kinase and block of phosphate incorporation into the enzyme. Eur. J. Biochem., 147, 33-40
11. Ushiroyama, T. Tanigawa, Y., Tsuchiya, M., Matsuura, R., Ueki, M., Sugimoto, O., and Shimoyama, M. (1985) Amino acid sequence of histone H1 at the ADP-ribose-accepting site and ADP-ribose-histone H1 adduct as an inhibitor of cAMP-dependent phosphorylation. Eur. J. Biochem., 151, 173-177
12. Ranieri-Raggi, M., Moir, A. J. G. and Raggi, A. (1985) Interaction with troponin T from white skeletal muscle restores in white skeletal muscle AMP deaminase those allosteric properties removed by limited proteolysis. Biochim. Biophys. Acta, 827,93-100
13. Simley, K. L., Berry, Jr. A. J. and Suelter, C. H. (1967) An improved purification, crystalization, and some properties of rabbit muscle 5-adenylic acid deaminase. J. Biol. Chem., 242, 2502-2506

14. Ranieri-Raggi, M. and Raggi, A. (1979) Regulation of skeletal muscle AMP deaminase. Effect of limited proteolysis on the activity of the rabbit enzyme. FEBS Letters, 102, 59-63
15. Laemmly, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature, 227, 680-685
16. Ranieri-Raggi, M. and Raggi, A. (1980) Skeletal muscle AMP-deaminase; Effects of limited proteolysis on the aggregation and regulatory properties. Ital. J. Biochem., 29, 218-222
17. Coffee, C. J. and Kofke, W. A. (1975) Rat muscle 5'-adenylic acid aminohydrolase. I. Purification and subunit structure, J. Biol. Chem., 250, 6653-6658
18. Riquelme, R. T., Burzio, L. O. and Koide, S. S. (1979) ADP-ribosylation of rat liver lysine-rich histone in vitro. J. Biol. Chem., 253, 3018-3028