Activation of an NAD:Arginine ADP-ribosyltransferase by Histone*

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An ADP-ribosyltransferase from turkey erythrocytes which utilizes proteins and low molecular weight guanidino compounds such as arginine and agmatine as ADP-ribosyl acceptors was stimulated by histones. The effect was specific in that cholera, a bacterial mono(ADP-ribosyl)transferase that increased adenylation activity in animal cells, was not activated by histones. With the erythrocyte enzyme, histones decreased the apparent $K_m$ values for arginine methyl ester and agmatine and increased the stability of the transferase to thermal denaturation. Activation of the transferase by histones was rapid, with a minimal delay observed upon addition of histones to a histone-free assay. Activation by histones was reversed upon dilution of a sample containing histones into an assay mixture free of histone. In the absence of histone, the transferase existed as a rapidly sedimenting species; in the presence of histone, the transferase sedimented as a protomer.

The transfer of the ADP-ribose moiety of NAD to specific proteins is utilized by both exogenous effectors and endogenous enzymes to control cellular metabolism (1). Among the exogenous agents, it appears, for example, that certain bacterial toxins such as cholera and diphtheria toxin exert their effects on cells by ADP-ribosylation (2, 3). Endogenous enzymes in animal species have been identified which catalyze poly- and mono(ADP-ribosylation). In the former case, poly(ADP-ribosyl) synthetase catalyzes the formation of long chain polymers of ADP-ribose in ribose-ribose linkage attached to acceptor proteins (1). A mono(ADP-ribosyl)transferase, which catalyzes the formation of mono(ADP-ribosyl) protein, has been reported in several tissues from different species (4-6). In addition to using protein as an acceptor, this transferase also catalyzes the ADP-ribosylation of arginine and certain other guanidino compounds (4, 7). The enzymatic and physical properties of the mono(ADP-ribosyl)transferase appear to be clearly distinct from those of the poly(ADP-ribose) synthetase. The synthetase is believed to be involved in the maintenance of chromatin structure and in DNA repair and replication (1). The purified enzyme is absolutely dependent on NAD for activity (8-12) and is stimulated by histones (8-11, 13, 14). The role of the mono(ADP-ribosyl)transferase is unclear; its activity appears to be independent of DNA (5). As noted recently, however, the transferase appears to exist in histone-dependent and histone-independent forms (15). Histones stimulate the dependent form of the enzyme >10-fold (15). In the present studies, we report that histones interact with both the histone-dependent and independent forms of the transferase; activation by histones appears to be rapid and reversible.

EXPERIMENTAL PROCEDURES

Methods—The ADP-ribosyltransferase was purified from the soluble fraction of turkey erythrocytes through the concanavalin A-agarose step as described (5). One major protein band was observed on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Protein was determined by the method of Lowry et al. (16).

NAD glycohydrolase and ADP-ribosyltransferase were assayed as described (17, 18). The standard assay contained 20 mM potassium phosphate (pH 7.0), 32.4 μM [carbonyl-$^3$H]NAD (40,000 cpm), 1 mg/ml ovalbumin, 6 mM amine, and other additions (e.g., histones) as indicated in a total volume of 0.3 ml. After addition of ADP-ribosyltransferase (~1 ng) and incubation at 30 °C for 30 min, two 0.1-ml samples were transferred to columns (0.5 × 2 cm) of AG 1-X2 for isolation of [carbonyl-$^3$H]nicotinamide (17). Data reported are means of values from duplicate assays.

Materials—Choleragen and dithiothreitol were obtained from Schwartz/Mann; [carbonyl-$^3$H]NAD (specific activity, 53 mCi/mmol) was from Amersham; AG 1-X2 was from Bio-Rad; ovalbumin, agmatine, ovalbumin, histone Type III, NAD, chymotryptsinogen, bovine serum albumin, polyarginine, lysyoxyme, and RNase were from Sigma; NaCl was from Fisher.

RESULTS

Activity of the erythrocyte ADP-ribosyltransferase assayed with subsaturating concentration of ADP-ribosyl acceptor was markedly increased by histone. The effect of histone was immediate (Fig. 1) and was rapidly reversible on transfer of the enzyme from assays containing histone (Fig. 2) to assays lacking histone (Fig. 3). The decrease in activity did not result from a loss of histone responsiveness due to inactivation in

Fig. 1. Effect of histones on the release of [carbonyl-$^3$H]nicotinamide from [carbonyl-$^3$H]NAD catalyzed by the erythrocyte ADP-ribosyltransferase. ADP-ribosyltransferase activity (○) was assayed with 1.4 μg of enzyme as described under "Methods" except that incubation time was varied as indicated. To some samples, 9 μg of histone (●) in 5 μl of water or as a control 5 μl of water (■) were added after 30 min. Histone concentration in the assay was ~30 μg/ml.

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**Fig. 2 (left).** Effect of histone on ADP-ribosyltransferase activity. Assays without (○) or with (■) histone, 20 μg/ml, containing standard assay components at the concentrations stated under "Methods" and 215 ng of transferase in a total volume of 1.5 ml were incubated at 30 °C. At the indicated times, two 0.1-ml samples were withdrawn to quantify [carbonyl-14C]nicotinamide release.

**Fig. 3 (right).** Reversal of the effect of histones on [carbonyl-14C]nicotinamide release from [carbonyl-14C]NAD catalyzed by the ADP-ribosyltransferase. Assay mixtures without (○, ●, ■) or with (□, △, ◼ histone set up as described in Fig. 2, except that [14C]NAD was omitted (32.4 μM NAD was present) were incubated for 5 min at 30 °C. Samples (50 μl) were then transferred to identical assay mixtures containing [carbonyl-14C]NAD without (○, □) or with histone (△, ◼), 2 μg/ml, or (■, △), 20 μg/ml. After the indicated times of incubation at 30 °C, two 0.1-ml samples were removed from duplicate assays for isolation of [carbonyl-14C]nicotinamide.

**Fig. 4.** Effect of NaCl and histones on ADP-ribosyltransferase activity. Standard assays contain the indicated concentration of NaCl without (○) or with (■) histone, 20 μg/ml.

The first assay, since the transferase responded to histone in the second mix (Fig. 3). In the presence of histone, 100 to 300 mM NaCl increased activity ~15% (Fig. 4). NaCl, in the absence of histone, increased activity in a concentration-dependent manner; with 300 mM NaCl, transferase activity was similar to that observed with histone without NaCl (Fig. 4). There was no apparent inhibition of the histone effect at intermediate NaCl concentration (Fig. 4).

Histone produced an activation of the transferase using limiting concentrations of agmatine which was similar to that produced by 200 mM NaCl (Fig. 5). An identical effect was observed with arginine methyl ester. The Ki for NAD in optimal concentrations of histone and NaCl was 15 μM. The

**Fig. 5 (left).** Effect of agmatine concentration on [carbonyl-14C]nicotinamide release with histone or NaCl. Standard assays contained the indicated concentration of agmatine and (■) no addition, (○) 200 mM NaCl, or (◆) histone, 20 μg/ml, and were initiated with transferase (1.43 ng).

**Fig. 6 (right).** Effect of histones on the stability of the erythrocyte ADP-ribosyltransferase in the presence and absence of NaCl. Samples of transferase (43 ng) were incubated (in duplicate) at 30 °C in a total volume of 0.3 ml containing 20 mM potassium phosphate (pH 7.0), 5% propylene glycol, and (◆) histone, 20 μg/ml, (□) 200 mM NaCl, or (○) histone plus NaCl. At the indicated times, samples (10 μl) were then assayed under standard conditions except that 75 mM arginine methyl ester replaced 6 mM agmatine. In contrast to the experiments described in Figs. 1–3, ovalbumin, which stabilized the enzyme, was absent from the initial incubation. A comparison of the relative effects of ovalbumin and histone on stability is given in Table II.

| Enzyme | -Histone | +Histone (20 μg/ml) |
|--------|----------|-------------------|
| 1. Transferase, 0.48 ng | 1.1 | 24.3 |
| Choleragen, 25 μg | 18.4 | 17.6 |
| 2. Choleragen, 25 μg | 64.2 | 62.3 |

**Table I**

Effect of histones on the activity of the erythrocyte ADP-ribosyltransferase

Assays were carried out under standard conditions except that 20 mM dithiothreitol was present and incubation was for 90 min. In addition, in Experiment 2, 400 mM (rather than 20 mM) potassium phosphate buffer, pH 7.0, was used.

| Protein added | ADP-ribosyltransferase activity (pmol min⁻¹) |
|---------------|---------------------------------------------|
| None | 12 (14) | 3 (3) |
| Histones | 91 (100) | 103 (100) |
| Ovalbumin | 18 (20) | 10 (10) |
| Bovine serum albumin | 35 (39) | 107 (104) |
| Chymotrypsinogen | 26 (29) | 81 (79) |
| Polyarginine | 1 (1) | 2 (2) |
| Lysozyme | 14 (15) | 5 (9) |
| Ribonuclease | 11 (12) | 2 (2) |

**Table II**

Effect of proteins on the stability of the erythrocyte ADP-ribosyltransferase

Samples of transferase (14.3 ng) were incubated (in duplicate) for 10 min at 30 °C in a total volume of 0.1 ml of 20 mM potassium phosphate (pH 7.0) containing NaCl and/or proteins as indicated. Two 10-μl samples were then assayed under standard conditions except that 75 mM arginine methyl ester replaced 6 mM agmatine. Activity of the enzyme that had not been incubated was 162 without added histone and 166 in the presence of histone, 0.67 μg/ml; all assays contained 75 mM arginine methyl ester. Numbers in parentheses indicate the percentage of the activity achieved with histone.

ADP-ribosyltransferase activity of choleragen was not enhanced by histone when assayed in the standard transferase assay (Experiment 1, Table I) or under choleragen assay conditions (Experiment 2, Table I).
Fig. 7. Effect of histone on sedimentation of the erythrocyte ADP-ribosyltransferase. Into SW 60 centrifuge tubes were layered 0.4 ml of 50% propylene glycol/20 mM potassium phosphate (pH 7.0)/0.2 M glycine, containing histone, 50 μg/ml, followed by 3.4 ml of 35% propylene glycol/20 mM potassium phosphate (pH 7.0)/0.2 M glycine, and finally 0.2 ml of 25% propylene glycol/20 mM potassium phosphate (pH 7.0)/0.2 M glycine, containing transferase (286 ng). Tubes were centrifuged at 304,700 × g for 90 min, after which 0.4-ml fractions were collected and samples (10 μl) were assayed under standard conditions except that 75 mM arginine methyl ester replaced 6 mM agmatine. At this concentration of arginine methyl ester, the transferase was maximally stimulated without a further histone requirement. The histones carried over from the gradient into the assay had no effect on recovery. The activity recovered in each case was >97%. Direction of sedimentation is from fraction 1 to 10. Percentage of total recovered activity in each fraction is recorded. A, no histone present in transferase solution, B, transferase solution contained histone, 20 μg/ml.

The transferase was rapidly inactivated during incubation at 30 °C with or without 300 mM NaCl and was stabilized by histone (Fig. 6, Table II). Other proteins, in the absence of salt, have much less or no effect on stability. Only histones of the proteins given in Table II were able to activate the transferase (15). In the presence of 300 mM NaCl, serum albumin was as effective as histone, and chymotrypsinogen provided considerable stabilization (Table II).

We have shown previously that the transferase is reversibly converted from a rapidly sedimenting oligomeric form(s) to a slowly sedimenting protomeric species by sodium chloride (19). In the absence of sodium chloride, histone also converts the transferase to a more slowly sedimenting species. The difference in sedimentation rate for the protomeric and oligomeric forms is quite large, and a special experimental design was required in order to observe both species under the same conditions (Fig. 7). The transferase, with and without histone, was layered on top of a propylene glycol gradient and sedimented at 304,000 × g for 90 min. The gradient, i.e., 50 and then 35% propylene glycol, and the presence of histone in the lower part of the gradient were used for stabilization and to keep the oligomeric form from pelleting. Under these conditions, activity remained near the meniscus in the presence of histone and sedimented to the bottom of the cell in the absence of histone (Fig. 7).

The sedimentation velocity experiments illustrated in Fig. 8 were designed to compare the molecular size of transferase generated by histone to that obtained by sodium chloride (19). The sedimentation boundary for transferase in histone and histone plus NaCl was similar to that of chymotrypsinogen (Fig. 8). It was previously shown that in salt alone the transferase sediments at a rate similar to that of chymotrypsinogen (M, = 25,300) and chromatographs on gel permeation columns with a Ks, similar to chymotrypsinogen (19).

DISCUSSION

Three effects of histone on the erythrocyte ADP-ribosyltransferase have been observed: rapid and reversible enhancement of catalytic activity, protection against thermal inactivation, and conversion of a rapidly sedimenting enzyme to a form that sediments at a rate comparable to chymotrypsinogen. All of these effects were maximal with histone concentrations of 20 μg/ml. Transferase activity was increased by 300 mM NaCl to almost the same level achieved with histone; both activators increased enzyme activity with either agmatine or arginine methyl ester as substrate. The Ks, for NaCl with both activators was identical. Histone and NaCl also had similar effects on the sedimentation velocity of the transferase (19).

Although both histone and salt caused dissociation of the oligomer, it appears that activation by salt did not preclude interaction with histones. The salt-activated enzyme was subjected to thermal denaturation at 30 °C; in the presence of histone alone or histone and salt, the enzyme was considerably more stable. Certain other proteins, which were not active in the absence of salt, also stabilized the transferase in the presence of salt. Conversion of the oligomers to protomeric forms by salt thus permitted histones and various other proteins to enhance transferase stability. In the absence of salt, stabilization by histones was somewhat specific; it occurred at low concentration and other nonhistone proteins were less effective. It was clearly less specific than activation which was only observed with histones (15).

Low concentrations of NaCl partially stimulated the transferase but did not block the activation by histone. If histone-ribosyltransferase complex formation were due to nonspecific electrostatic interactions, increasing the concentration of inorganic salt should, as a result of charge shielding, cause a decrease in histone stimulation. No such partial reduction of histone-stimulated ADP-ribosyltransferase activity was ap-
Thus, the physiological importance of this
remains to be established.

The erythrocyte mono(ADP-ribosyl)transferase possesses
striking similarities and differences with the poly(ADP-ri-
bose)synthetase, the enzyme that is believed to play a role in
DNA repair and replication (1). The transferase is a much
smaller protein with a subunit M, ~28,000 (5) compared to
weights of 63,000–130,000 for the synthetases from diverse
sources (8–10, 12, 14). The transferase to date has not been
shown to catalyze poly(ADP-ribosylation); the synthetase is
currently believed to catalyze both the initial mono(ADP-
ribosylation) of acceptor, followed subsequently by chain elon-
gation (11, 14, 20). The acceptor amino acid for ADP-ribose
appears to be an arginine for the transferase (4, 7). The
synthetase appears to modify the carboxyl moieties of specific
glutamate residues and a COOH-terminal lysine (21–24). The
synthetase is absolutely dependent on DNA for activity (8–
12) and can exist in histone-dependent and histone-independ-
ent forms (11). The transferase does not appear to require
DNA (5), but it too exists in histone-dependent and independ-
ent forms. Thus, it would appear that in vitro histones may
activate two different enzymes that catalyze ADP-ribose
transfer. Since NaCl activates the transferase at physiological
concentrations, the in vitro function of histone is unclear.
Thus, the physiological importance of this in vitro regulation
remains to be established.

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