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Amino Acid Specific ADP-Ribosylation: Specific NAD:Arginine Mono-ADP-Ribosyltransferases Associated with Turkey Erythrocyte Nuclei and Plasma Membranes

Robert E. West, Jr.,* and Joel Moss

Laboratory of Cellular Metabolism, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892

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ABSTRACT: Turkey erythrocytes contain NAD:arginine mono-ADP-ribosyltransferases which, like cholera toxin and *Escherichia coli* heat-labile enterotoxin, catalyze the transfer of ADP-ribose from NAD to proteins, to arginine and other low molecular weight guanidino compounds, and to water. Two such ADP-ribosyltransferases, A and B, have been purified from turkey erythrocyte cytosol. To characterize further the class of NAD:arginine ADP-ribosyltransferases, the particulate fraction was examined; 40% of erythrocyte transferase activity was localized to the nucleus and cell membrane. Transferase activity in a salt extract of a thoroughly washed particulate preparation was purified 36 000-fold by sequential chromatography on phenyl-Sepharose, (carboxymethyl)cellulose, concanavalin A–Sepharose, and NAD–agarose. Subsequent DNA–agarose chromatography separated two activities, termed transferases C and A′, which were localized to the membrane and nucleus, respectively. Transferase C, the membrane-associated enzyme, was distinguished from the cytosolic enzymes by a relative insensitivity to salt and histone; transferase C was stimulated 2-fold by 300 mM NaCl in contrast to a 20-fold stimulation of transferase A and a 50% inhibition of transferase B. Similarly, histones, which stimulate transferase A 20-fold, enhanced transferase C activity only 2-fold. Transferase A′, the nuclear enzyme, was retained on DNA–agarose. It was similar to transferase A in salt and histone sensitivity. Gel permeation chromatography showed slight molecular mass differences among the group of enzymes: A, 24 300 daltons (Da); B, 32 700 Da; C, 26 000 Da; and A′, 25 500 Da. The affinities of transferase C for NAD and agmatine were similar to those of the cytosolic transferases A and B. Thus, mono-ADP-ribosyltransferases of the turkey erythrocyte occur as a group of similar enzymes whose multiple forms can be distinguished by their specific intracellular localization and their regulatory properties.

Mono-ADP-ribosylation, the transfer of the ADP-ribose moiety from NAD to specific protein substrates, is a mechanism of action common to several bacterial toxins and bacteriophage enzymes, some of which effect profound changes in cellular metabolism (Honjo & Hayaishi, 1973; Iglewski et al., 1977, 1978; Moss & Vaughan, 1977; Gill & Meren, 1978; Moss et al., 1979; Katada & Ui, 1982; Goff, 1974; Pesce et al., 1976). Among these are diphtheria toxin and *Pseudo-*

* Address correspondence to this author.

monas exotoxin A, which ADP-ribosylate elongation factor 2, thereby inhibiting cellular protein synthesis (Honjo & Hayaishi, 1973; Iglewski et al., 1977), cholera toxin, which ADP-ribosylates G_s, the guanine nucleotide binding protein that couples stimulatory receptors to adenylate cyclase, resulting in persistent activation of adenylate cyclase (Gill & Meren, 1978; Cassell & Pfeuffer, 1978; Johnson et al., 1978; Northup et al., 1980), and pertussis toxin, which ADP-ribosylates G_i, the guanine nucleotide binding protein that couples inhibitory receptors to adenylate cyclase, interfering with receptor-mediated inhibition of adenylate cyclase (Katada & Ui, 1981, 1982; Bokoch et al., 1983).

Certain toxins have been characterized on the basis of the amino acid to which they transfer ADP-ribose. Thus, diphtheria toxin and *Pseudomonas* exotoxin A transfer ADP-ribose to diphthamide, a modified histidine residue which occurs in elongation factor 2 (Van Ness et al., 1980); cholera toxin and *Escherichia coli* heat-labile enterotoxin ADP-ribosylate arginine (Moss & Vaughan, 1977; Moss et al., 1979; Van Dop et al., 1984); pertussis toxin ADP-ribosylates a cysteine residue (West et al., 1985).

The physiologic importance of ADP-ribosylation in eukaryotes has long been recognized in the nuclear poly(ADP-ribose) polymerase which forms long branching chains of ADP-ribose attached to glutamic acid residues of nuclear proteins and is apparently involved in chromatin maintenance (Ueda et al., 1982). More recently, defining the sites of toxin-catalyzed ADP-ribosylation has led to the identification of mono-ADP-ribosyltransferases endogenous to eukaryotes. An enzyme that ADP-ribosylates the diphthamide of elongation factor 2 has been identified in cultured baby hamster kidney cells, bovine liver, and rabbit reticulocytes (Lee & Iglewski, 1984; Sitikov et al., 1984). Enzymes that ADP-ribosylate arginine have been identified in turkey erythrocyte cytosol, hen liver nuclei, rat liver mitochondria, rabbit skeletal muscle, and *Xenopus* tissues (Moss et al., 1980; Yost & Moss, 1983; Tanigawa et al., 1984; Richter et al., 1983; Soman et al., 1984; Godeau et al., 1984). Moreover, ADP-ribosylation of arginine residues appears to be a reversible modification of proteins; an enzyme from SVT2 mouse fibroblasts and turkey erythrocytes catalyzes hydrolysis of the ribosylarginine bond, regenerating the arginine residue with an intact ADP-ribose acceptor site (Smith et al., 1985; Moss et al., 1985).

In the turkey erythrocyte, two distinct NAD:arginine mono-ADP-ribosyltransferases, termed A and B, have been purified from cytosol (Moss et al., 1980; Yost & Moss, 1983). The reported purification of a nuclear NAD:arginine mono-ADP-ribosyltransferase from hen liver (Tanigawa et al., 1984), an enzyme distinct from the other arginine transferases as well as the nuclear poly(ADP-ribose) polymerase, induced us to examine particulate preparations from turkey erythrocytes, the study of which should be relatively straightforward as turkey erythrocytes, like other nonmammalian, vertebrate erythrocytes, comprise only nuclei, plasma membranes, and cytosol (Zentgraf et al., 1971). We report here the localization of a distinct NAD:arginine mono-ADP-ribosyltransferase, C, to turkey erythrocyte membranes, and another, A′, superficially similar to the cytosolic transferase A but clearly localized to the turkey erythrocyte nucleus.

Materials and Methods

Materials. Nicotinamide [U-^{14}C]adenine dinucleotide (266 mCi/mmol) was purchased from Amersham; NAD, agmatine sulfate, ovalbumin, and methyl α-D-glucoside were from Sigma; Nonidet P40 was from Bethesda Research Laboratories; phenyl-Sepharose, Sephadex G-25, and concanavalin A–Sepharose were from Pharmacia; (carboxymethyl)cellulose was from Whatman; NAD–agarose (type 1) and DNA–agarose were from P-L Biochemicals; the TSK 2000 column was from Altex; the Zorbax SAX column was from Du Pont; and AG1 × 2 (200–400 mesh, Cl^- form) was from Bio-Rad. Turkey blood was obtained from the National Institutes of Health for localization studies and from Pel-Freez for enzyme purification and characterization.

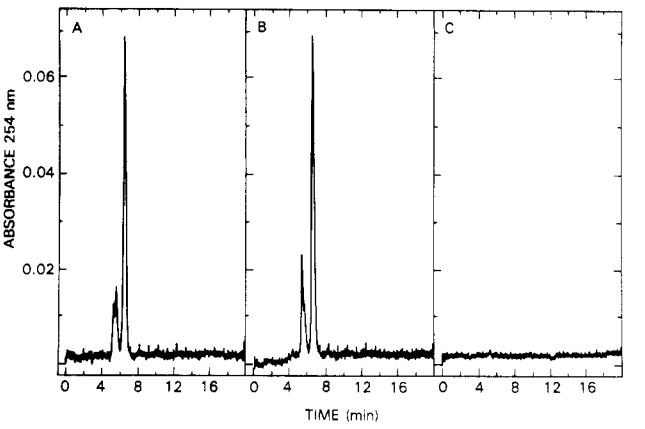

FIGURE 1: Reaction product identification. The product was identified as described under Materials and Methods. Chromatograms are from incubations containing (A) transferase A plus 30 mM agmatine, (B) transferase C plus 30 mM agmatine, and (C) transferase C without agmatine. The retention time for the major product (80%) formed by transferases A, C, and A′ was 6.5 min. The minor product formed did not correspond to any of the adenine-containing standards that were run.

ADP-Ribosyltransferase Assay. NAD:arginine mono-ADP-ribosyltransferase activity was assayed by using agmatine as the ADP-ribose acceptor (Moss & Vaughan, 1984). Each assay contained 30 μM [*adenine*-U-^{14}C]NAD (~40000 cpm), 50 mM potassium phosphate, pH 7, 0.3 mg of ovalbumin with or without 2 mM agmatine sulfate, and 300 mM NaCl as indicated in a final volume of 0.3 mL. Thirty-minute incubations at 30 °C were initiated with addition of [*adenine*-U-^{14}C]NAD. A 0.1-mL sample of each reaction mixture was applied to an AG1 × 2 column (0.5 × 3.5 cm); the product was eluted with 5 mL of water, and radioactivity was assayed by liquid scintillation counting. A unit of activity is equivalent to the amount of enzyme that forms 1 μmol of ADP-ribosylagmatine/min under these conditions.

Product Identification. A slightly modified reaction mixture (100 μM [*adenine*-U-^{14}C]NAD (10^6 cpm) and 30 mM agmatine) containing 10^{-4} unit of transferase activity was incubated for 90 min, and then 0.28 mL was applied to the AG1 × 2 column. Product was eluted with five 1-mL water washes. Fifty microliters of the fraction containing peak radioactivity (~40% of eluted radioactivity) was applied to a Du Pont Zorbax SAX column (0.46 × 25 cm) in a mobile phase of 40 mM potassium phosphate, pH 4.5 (flow rate 1 mL/min, ambient temperature). The absorbance at 254 nm was monitored, and 1-mL fractions were collected for liquid scintillation counting.

The chromatographic profile of Figure 1 shows that the product of the transferase C catalyzed reaction was agmatine dependent and had the same elution time as ADP-ribosylagmatine formed by transferase A. The product of the transferase A′ catalyzed reaction was likewise identified.

Localization of Particulate Mono-ADP-Ribosyltransferases. Two hundred milliliters of freshly drawn, heparinized turkey blood was centrifuged 10 min at 1500*g*, and the plasma and buffy coat were aspirated. Cells were washed twice with an equal volume of 0.25 M sucrose/25 mM tris(hydroxy-

methyl)aminomethane hydrochloride (Tris-HCl),[1] pH 7.5/5 mM $MgCl_2$ and centrifuged each time for 10 min at 1500*g*. Cells were suspended for 0.5 h in 3 volumes of ice-cold 10 mM Tris-HCl, pH 7.5/5 mM $MgCl_2$ and then disrupted by a Polytron (Brinkmann)(PTA 10S probe, setting 5.5, 30 s). The homogenate was centrifuged 10 min at 1500*g*. The pellet was used as nuclei (1.85 g of protein, 456 mg of DNA, 51% of total homogenate DNA). The 1500*g* supernatant was centrifuged 10 min at 5000*g*. This supernatant was then centrifuged 10 min at 50 000*g*. The 50000*g* pellet contained cell membranes (390 mg of protein, 8 mg of DNA). Each pellet was washed 5 times with 10 mM Tris-HCl, pH 7.5/5 mM $MgCl_2$, disrupted briefly each time with a Polytron, and centrifuged. The washed pellets were extracted by a Polytron (setting 5, 45 s), nuclei in 20 mL and membranes in 10 mL of ice-cold 0.3 M NaCl/50 mM Tris-HCl, pH 7.5/50 mM sodium bisulfite/10 mM mercaptoethanol/10% glycerol (v/v) (0.3 M NaCl/extraction buffer). Extracts were centrifuged 10 min at 50000*g*. One milliliter of each extract was applied to a column of DNA–agarose (0.75 × 2.2 cm). Transferase C was eluted with four 1-mL portions of 0.3 M NaCl/extraction buffer followed by five 1-mL portions of 1 M NaCl/extraction buffer to elute transferase A′. DNA–agarose fractions were assayed in the absence and presence of 300 mM NaCl. The activity tabulated is salt-stimulated.

Substantially purified nuclei (335 mg of protein, 652 mg of DNA, 100% of cell DNA) were prepared with the following modifications. The twice-washed 1500*g* cell pellet was lysed in approximately 4 volumes of 10 mM Tris-HCl, pH 7.5/5 mM $MgCl_2$/10 mM NaCl/0.5% Nonidet P40 and washed 3 times more in the same buffer with centrifugation (10 min, 3000*g*) each time. Nuclei were washed once with 0.25 M sucrose/25 mM Tris-HCl, pH 7.5/5 mM $MgCl_2$ and then centrifuged 1 h at 37000*g* through 2.4 M sucrose/25 mM Tris-HCl, pH 7.5/5 mM $MgCl_2$. Thereafter, they were frozen and extracted as above in 10 mL of 0.3 M NaCl/extraction buffer.

Purification of Particulate Mono-ADP-Ribosyltransferases. Five liters of turkey blood was centrifuged at 3000*g*, the plasma and buffy coat were aspirated, and the cells were washed twice in equal volumes of 0.25 M sucrose/25 mM Tris-HCl, pH 7.5/5 mM $MgCl_2$. The 1.5 L of packed, washed cells was suspended in an equal volume of ice-cold 10 mM Tris-HCl, pH 7.5/5 mM $MgCl_2$ for 20 min and then disrupted for 1 min at high speed in a commercial Waring Blendor. The homogenate was centrifuged at 25000*g*, and sedimented material was washed 6 times with lysis buffer. Enzyme activity was extracted from the washed particulate material (500 mL) in four successive 500-mL volumes of 0.5 M NaCl/extraction buffer in a Waring Blendor at high speed. This material was pooled, the salt concentration adjusted to 1.2 M, and the material stirred overnight with 180 mL of phenyl-Sepharose. The phenyl-Sepharose was washed in a fritted glass funnel, first with 10 bed volumes of 50 mM sodium phosphate, pH 7/1 M NaCl, next with 2 bed volumes of 50 mM sodium phosphate, pH 7, and finally with 0.5 bed volume of 50% propylene glycol/50 mM sodium phosphate, pH 7. Enzyme activity was eluted with 7.5 bed volumes of 50% propylene glycol/50 mM sodium phosphate, pH 7. This material was applied to 700 mL of (carboxymethyl)cellulose which was then washed with 3.5 L of 50 mM sodium phosphate, pH 7. One-half bed volume of 1 M NaCl/50 mM sodium phosphate, pH 7, was applied, and then enzyme was eluted with another bed volume of 1 M NaCl/50 mM sodium phosphate, pH 7. After concentration on 10 mL of phenyl-Sepharose, the activity was applied to 40 mL of concanavalin A–Sepharose in a fritted glass funnel, the gel washed with 3.5 bed volumes of 50 mM sodium phosphate, pH 7/25% propylene glycol, and the activity eluted with 230 mL of 0.4 M methyl α-D-glucoside/0.4 M NaCl/50 mM sodium phosphate, pH 7/25% propylene glycol. This material was applied to a column of NAD–agarose (1.5 × 3 cm) at a linear flow rate of 18 cm/h. The column was washed with 20 bed volumes of 50 mM sodium phosphate, pH 7/20% propylene glycol, and the enzyme activity was eluted with 1.5 M $MgCl_2$/50 mM sodium phosphate, pH 7/20% propylene glycol collected in 5-mL fractions. After buffer exchange on Sephadex G-25 (2.5 × 17.5 cm), the material (34 mL) was applied to DNA–agarose (1.5 × 4.5 cm) at a linear flow rate of 26 cm/h in 0.3 M NaCl/extraction buffer, and 10-mL fractions were collected. Another 40 mL of 0.3 M NaCl/extraction buffer was applied, followed by 40 mL of 0.6 M NaCl/extraction buffer and 30 mL of 1 M NaCl/extraction buffer. Fractions 1–4 were pooled as transferase C and concentrated on phenyl-Sepharose; fractions 7–9 were pooled as transferase A′ and likewise concentrated.

Miscellaneous. Protein was assayed according to Lowry et al. (1951), with bovine serum albumin as standard, and DNA according to Burton (1956).

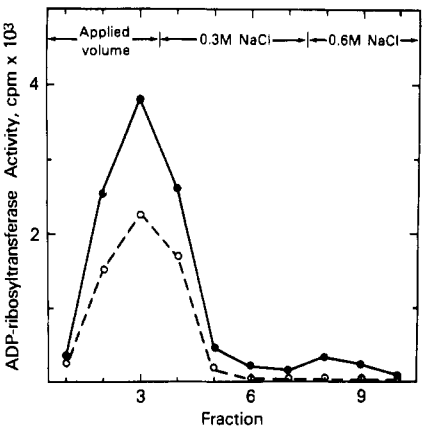

FIGURE 2: DNA–agarose elution profile. The profile shown is for the 36 000-fold purified enzyme as described under Materials and Methods and summarized in Table II. Results were similar with crude extracts of particulate preparations. Basal activity (5 mM NaCl for fractions 1–7, 10 mM NaCl for fractions 8–10) (○); activity assayed with 300 mM added NaCl (●). Similar results were obtained with 100 μg of histone IIA/mL instead of NaCl.

RESULTS

Identification of Particulate Mono-ADP-Ribosyltranferases. Particulate preparations of turkey erythrocytes, pink but substantially free of cytosol, when extracted with 0.3 M NaCl/50 mM Tris-HCl, pH 7.5/50 mM sodium bisulfite/10 mM mercaptoethanol/10% glycerol, yielded NAD–agmatine mono-ADP-ribosyltransferase activity that was resolved by DNA–agarose chromatography into two peaks (Figure 2). Activity eluted from DNA–agarose with 0.3 M NaCl was stimulated <100% by addition to assays of 0.3 M NaCl or histone (100 μg/mL); activity eluted with 0.6 M NaCl was increased 5–20-fold by 300 mM NaCl or histone. The former activity peak was designated transferase C and the latter transferase A′.

[1] Abbreviations: SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; extraction buffer, 50 mM Tris-HCl, pH 7.5 (25 °C)/50 mM sodium bisulfite/10 mM mercaptoethanol/10% glycerol (v/v) containing sodium chloride as indicated; lysis buffer, 10 mM Tris-HCl, pH 7.5 (25 °C)/5 mM $MgCl_2$; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; Da, dalton(s).

Table I: Localization of Particulate Turkey Erythrocyte Mono-ADP-Ribosyltransferases[a]

	total act. (milliunits)		milliunits/mg of protein		milliunits/mg of DNA	
	A′	C	A′	C	A′	C
membranes	12	83	0.031	0.21	1.5	10
nuclei	530	400	0.29	0.22	1.2	0.88
detergent-washed nuclei	124	6	0.37	0.018	0.19	0.009

[a] Nuclei and membranes were prepared as described under Materials and Methods. ADP-ribosyltransferase activity was extracted from each fraction with 0.3 M NaCl and applied to DNA–agarose. Activity that was eluted from DNA–agarose with 0.3 M NaCl and which was slightly (<2-fold) stimulated by salt was defined as transferase C; activity that was eluted with 1 M NaCl and which was stimulated 5–20-fold by 0.3 M NaCl was defined as transferase A′.

Localization. Specific intracellular localization of transferases A′ and C was shown by fractionation of erythrocytes into membranes and nuclei (Table I). Transferase A′ was clearly a nuclear enzyme. Ninety-eight percent was localized to the nucleus, and its activity relative to DNA was the same in membrane and nuclear fractions whereas, relative to protein, the activity was enriched 9-fold in nuclei over membranes. Conversely, transferase C activity, relative to DNA, was enriched 11-fold in membranes over nuclei. Relative to protein, its activity was the same in membranes and nuclei. The data are consistent with transferase C being a membrane-associated enzyme.

Yet, because most transferase C activity (83%) was associated with the nuclear fraction, detergent-washed nuclei were prepared in order to remove contaminating plasma membranes (Zentgraf et al., 1969). The detergent wash reduced total nuclear-associated transferase C activity 67-fold and its specific activity 97-fold relative to DNA and 12-fold relative to protein. The effect of the detergent wash on nuclear transferase A′ activity was much less pronounced; its total activity was reduced just 4-fold and its specific activity relative to DNA 6-fold while its specific activity relative to protein increased 1.3-fold. Again, the data are consistent with a nuclear localization for transferase A′ and a membrane association for transferase C.

Purification. The two enzymes were substantially copurified (36 000-fold) before being resolved on DNA–agarose (Table II, Figure 2). First, particulate material was freed of cytosolic proteins by an exhaustive series of hypotonic washes. The cytosol itself contained 47 units of transferase activity, the washes another 20.7 units. The last two washes combined contained less than 1% (0.6 unit) of the cytosolic transferase activity. Subsequent elution of the particulate material with 0.5 M NaCl/extraction buffer yielded a total of 46 units of transferase activity, or 40% of the total cellular transferase activity, with a specific activity approximately 10-fold higher than that of cytosol; only 1% of the total activity remained in the pellet. The two particulate activities copurified through phenyl-Sepharose, (carboxymethyl)cellulose, concanavalin A–Sepharose, and NAD–agarose. Transferase C was eluted from DNA–agarose with 0.3 M NaCl and transferase A′ with 0.6 M NaCl to give a 54 000-fold enrichment of specific activity for transferase C.

Characterization. The apparent molecular weights of the partially purified enzymes were determined by gel permeation chromatography along with the purified transferases A and B. Retention times and apparent molecular weights, respectively, were 34.0 min and 25 500 for transferase A′ and 33.7 min and 26 000 for transferase C. Transferase A was eluted at 35.4 min (24 300 Da) and transferase B at 32.3 min (32 700 Da). Values previously determined by SDS–PAGE were 28 300 Da for transferase A and 32 000 Da for transferase B (Moss et al., 1980; Yost & Moss, 1983).

Kinetic parameters were determined for transferase C by assuming Michaelis–Menten kinetics (Figure 3). Its K_m for NAD was 15 μM; its K_m for agmatine was 2 mM. These are similar to the kinetic constants determined for transferases A and B (Moss & Vaughan, 1984).

Discussion

The NAD:arginine mono-ADP-ribosyltransferases previously purified from turkey erythrocyte cytosol account for 60% of the activity assayable in vitro in the present study. While one cannot rule out the possibility of their leakage from nuclei, the preponderantly extranuclear localization of mono-ADP-ribosylproteins (Adamietz et al., 1981) supports the inference that transferases A and B are cytosolic enzymes. In addition

Table II: Purification of Particulate Mono-ADP-Ribosyltransferases[a]

stage	act. (units)	protein (mg)	sp act. (milliunits/mg)	yield (%)	purification (x-fold)
homogenate	59	434000	0.14		
cytosol	47	292000	0.16		
resuspension 1	50	138000	0.28		
wash 1	8.6	39000	0.22		
wash 2	6.6	25000	0.26		
wash 3	4.0	13000	0.31		
wash 4	0.9	5900	0.15		
wash 5	0.3	1600	0.18		
wash 6	0.3	765	0.32		
resuspension 2	33	25000	1.3		9
extract 1	17	3900	4.4		
extract 2	10	2600	3.8		
extract 3	9.0	3200	2.8		
extract 4	8.4	17000	0.50		
resuspension 3	1.0	1800	0.55		
pooled extracts	46	26700	1.7	97	12
phenyl-Sepharose	29	367	79	61	564
(carboxymethyl)cellulose	26	100	260	55	1857
concanavalin A–Sepharose	9.4	16	590	20	4214
NAD–agarose	2.6	0.51	5100	5.5	36429
DNA–agarose					
transferase C	0.92	0.12	7600	2.0	54286
transferase A′	0.072			0.16	

[a] Turkey erythrocytes were hypotonically lysed and mechanically disrupted. Cytosol was removed with six washes, following which particulate transferases were salt-extracted and purified as described under Materials and Methods.

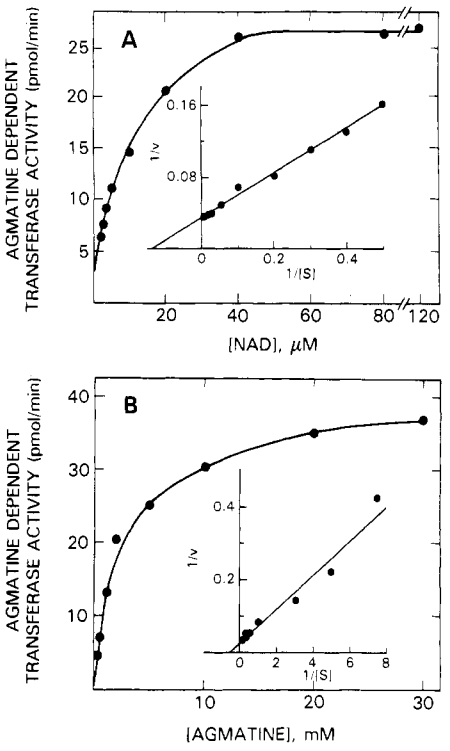

FIGURE 3: Reaction velocity vs. substrate concentration for transferase C. A 5600-fold purified preparation which had been through all stages except NAD–agarose was used for these assays. (A) NAD concentration dependence in the presence of 10 mM agmatine. Inset units are as follows: abscissa, μM^{-1}; ordinate, $pmol^{-1}$·min. (B) Agmatine concentration dependence in the presence of 150 μM NAD. Inset units are as follows: abscissa, μM^{-1}; ordinate, $pmol^{-1}$·min. K_m's, determined from the double-reciprocal plots, were 15 μM for NAD and 2 mM for agmatine.

to these enzymes purified from cytosol, we have now localized specific NAD:arginine mono-ADP-ribosyltransferases to the turkey erythrocyte membrane and nucleus. The membrane-associated transferase C is distinguished from the cytosolic enzymes by a relative insensitivity to salt or histones whereas cytosolic transferase A is stimulated 20-fold by salt or histones and cytosolic transferase B is inhibited by salt and unaffected by histones. Superficially, the nuclear transferase A′ is similar to the cytosolic transferase A in that both enzymes are stimulated by salt or histones. In this respect, transferase A′ differs from the nuclear mono-ADP-ribosyltransferase of hen liver nuclei, which is inhibited by salt (Tanigawa et al., 1984). That transferase A′ ADP-ribosylates low molecular weight guanidino compounds and its activity is insensitive to DNA (data not shown) distinguish it from the poly(ADP-ribose) polymerase isolated from the nuclei of numerous cell types (Ueda et al., 1982). The distinctions between transferases A′ and A are based on their chromatographic behavior. To wit, the relatively mild conditions employed to elute transferase A from NAD–agarose—50% propylene glycol—are insufficient to elute transferase A′ which requires high concentrations of $MgCl_2$. In addition, the two transferases consistently show slightly different retention times on gel permeation columns. The chromatographic differences coupled with the difference in subcellular localization are consistent with the hypothesis that A and A′ are distinct transferases.

The occurrence of multiple mono-ADP-ribosyltransferases is consistent with diverse functions for mono-ADP-ribosylation in the cell. From the association of different enzymes with specific structures, an allocation of particular functions to individual enzymes may be inferred. How they are regulated and to what extent they are related remain to be determined. An example of an enzyme that occurs in membrane-bound and soluble forms is dopamine β-hydroxylase (Sabban et al., 1983a). Treatment of PC12 cells with nerve growth factor, dexamethasone, or dibutyryl-cAMP causes a shift from the higher molecular weight membrane-bound form to the lower molecular weight cytosolic form (Sabban et al., 1983b). In erythrocytes, glyceraldehyde-3-phosphate dehydrogenase and aldolase occur in cytosol and in association with the plasma membrane, with differences in activity dependent upon location (Tsai et al., 1982; Murthy et al., 1981). Purification of multiple erythrocyte transferases should permit study of their regulation.

The localization of mono-ADP-ribosyltransferases to the nuclei of two cell types raises questions about the relationship of mono- to poly-ADP-ribosylation in the nucleus, whether, for instance, in vivo the mono-ADP-ribosyltransferase may initiate a chain at arginine that poly(ADP-ribose) polymerase, which normally initiates chains at glutamate residues (Riquelme et al., 1979; Ogata et al., 1980; Williams et al., 1984), then extends. Nonenzymatically formed Schiff bases between ADP-ribose and histone H1 serve as substrates for elongation by rat liver poly(ADP-ribose) polymerase (Ueda et al., 1979). Likewise, mono-ADP-ribosylated histone adducts formed by the chicken liver nuclear mono-ADP-ribosyltransferase serve in vitro as sites for elongation by chicken liver poly(ADP-ribose) polymerase (Tanigawa et al., 1984).

The distinction between poly(ADP-ribose) amino acid linkages based on sensitivities to neutral hydroxylamine treatment (Hilz, 1981) may be explained by different transferases initiating ADP-ribosylation. One extremely labile bond is characteristic of a glutamate linkage as formed by poly(ADP-ribose) polymerase; the other more stable bond is typical of the linkage formed by the mono-ADP-ribosyltransferases. Variations in the occurrence of the two bonds described during different stages of development and cell differentiation may be due to an interplay of mono-ADP-ribosyltransferases and poly(ADP-ribose) polymerase. The identification of two distinct nuclear NAD–arginine mono-ADP-ribosyltransferases, one in turkey erythrocytes as described here and the other in chicken liver nuclei (Tanigawa et al., 1984), makes this an increasingly likely possibility.

Registry No. NAD:arginine mono-ADP-ribosyltransferase, 81457-93-4.

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Identifications of the True Carbon-13 Nuclear Magnetic Resonance Spectrum of the Stable Intermediate II in Bacterial Luciferase†

Jacques Vervoort,‡ Franz Muller,*,‡ John Lee,§ Willy A. M. van den Berg,‡ and Chrit T. W. Moonen‡
Department of Biochemistry, Agricultural University, NL-6703 BC Wageningen, The Netherlands, and Department of Biochemistry, The University of Georgia, Athens, Georgia 30602

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ABSTRACT: Intermediate II in bacterial luciferase, formed in a reaction of luciferase with $FMNH^-$ and O_2, has been reinvestigated by ^{13}C NMR spectroscopy with ^{13}C-enriched FMN derivatives. It is shown that the previously published spectrum of the intermediate by Ghisla et al. [Ghisla, S., Hastings, J. W., Favaudon, V., & Lhoste, J.-M. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 5860–5863] does not represent the intermediate but is due to a contamination contained in commercially available [$^2H_6$]ethylene glycol. Relaxation studies show that the resonance line due to the byproduct of [$^2H_6$]ethylene glycol is easier to observe at low temperature than at room temperature, explaining fully the spectral properties of the published artifact. The true C(4a) resonance is found at 82.5 ppm, and this is interpreted as due to considerable sp^2 hybridization at this position, indicating an almost planar configuration by comparison with a model compound.

Bacterial luciferase is a flavoprotein utilizing riboflavin 5′-phosphate (FMN)[1] as a prosthetic group and catalyzing the oxidation of long-chain aliphatic aldehydes. The reaction is accompanied by emission of light. In the course of the bioluminescence reaction, several intermediates are formed, and their involvement in the reaction is still under dispute [e.g., Lee (1985) and Ziegler & Baldwin (1981)]. However, one

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* Author to whom correspondence should be addressed.
‡ Agricultural University.
§ The University of Georgia.

[1] Abbreviations: NMR, nuclear magnetic resonance; TMS, tetramethylsilane; FMN, oxidized riboflavin 5′-phosphate; $FMNH_2$ and $FMNH^-$, two-electron-reduced riboflavin 5′-phosphate in the neutral and anionic state, respectively.

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