

NAD Glycohydrolase and ADP-Ribosyltransferase Activities Are Intrinsic to the A₁ Peptide of Cholera^{*}

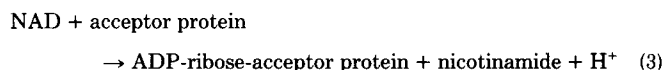
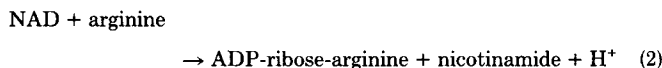
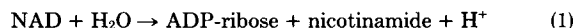
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In prior reports (Moss, J., Manganiello, V. C., and Vaughan, M. (1976) *Proc. Natl. Acad. Sci. U. S. A.* 73, 4424-4427), it was demonstrated that cholera^{*} preparations possessed NAD glycohydrolase activity. Tait and Van Heyningen (1978) *Biochem. J.* 174, 1059-1062) have recently proposed that the activity observed in these preparations was due to a contaminating enzyme and was not an intrinsic activity of cholera^{*}. The absence of an NAD glycohydrolase activity in cholera^{*} preparation would imply that nicotinamide release from NAD requires a ternary complex of NAD, acceptor protein, and toxin and that, in contrast to diphtheria toxin and *Pseudomonas* exotoxin A, cholera^{*} cannot use water as an ADP-ribose acceptor. Since the ability to hydrolyze NAD is critical to understanding the mechanism of the toxin-catalyzed reaction, we have re-examined the question using purified cholera^{*} peptides. In agreement with our prior observations, the purified A₁ peptide of cholera^{*}, but not the A₂ or B, catalyzed the hydrolysis of NAD to ADP-ribose and nicotinamide and the NAD-dependent ADP-ribosylation of arginine. The NAD glycohydrolase activity co-chromatographed with the A₁ peptide, the ADP-ribosyltransferase activity, and the ability to activate rat liver adenylate cyclase. The *K_m* for NAD was similar for the holotoxin and A₁. The turnover number of A₁ was similar to that of the intact toxin which contains one A₁ per molecule. In contrast to the holotoxin or its A subunit, the A₁ peptide did not exhibit a lag in reaction rate or a thiol requirement for activity. The enzymatic activity of the A₁ peptide was inhibited by sodium dodecyl sulfate as was its ability to activate adenylate cyclase. These results support the conclusion that the A₁ peptide of cholera^{*} possesses NAD glycohydrolase activity and can activate the ribosyl-nicotinamide bond of NAD in the absence of an acceptor protein.

Cholera^{*} appears to exert its effects on cells through the NAD-dependent activation of adenylate cyclase (1-4). A possible role for NAD as a substrate in an ADP-ribosylation reaction was suggested by the initial observations that cholera^{*} will catalyze NAD hydrolysis (Reaction 1) (5, 6) and the ADP-ribosylation of arginine (Reaction 2) (7); it has since been shown that cholera^{*} will also ADP-ribosylate many proteins (Reaction 3) (8-12). In a recent report, Tait and Van Heyningen (13) have stated that the NAD glycohydrolase activity found in cholera^{*}



preparations is due to a contaminating enzyme. The ability of cholera^{*} to catalyze NAD hydrolysis, however, is of importance to an understanding of the mechanism of toxin action. The presence of NAD glycohydrolase activity demonstrates 1) that cholera^{*} can use water as an ADP-ribose acceptor and 2) that the toxin can activate the ribosyl-nicotinamide bond of NAD in the absence of an acceptor protein.

Since work on two other NAD-dependent bacterial toxins, diphtheria toxin (14) and *Pseudomonas* exotoxin A (15), is consistent with the hypothesis that NAD hydrolysis occurs in the absence of a protein acceptor and since our previous findings were consistent with the A subunit of cholera^{*} possessing an intrinsic NAD glycohydrolase activity (5), we examined the ability of purified peptides from cholera^{*} to activate adenylate cyclase and catalyze NAD hydrolysis. The present investigation supports our previous findings that NAD glycohydrolase activity is intrinsic to the A subunit and now, more specifically, to the A₁ peptide of cholera^{*}.

EXPERIMENTAL PROCEDURES

Materials—Cholera^{*} and dithiothreitol were purchased from Schwarz/Mann; arginine methyl ester di-HCl, ovalbumin, and NAD from Sigma; AG 1-X2 (200 to 400 mesh) from Bio-Rad; [carbonyl-¹⁴C]NAD (50 mCi/mmol), [adenine-U-¹⁴C]NAD (280 mCi/mmol), and L-[³H]arginine (11 mCi/mmol) from Amersham/Searle. [α-³²P]-ATP was from ICN.

Assays—NAD glycohydrolase and ADP-ribosyltransferase assays were performed as described previously (5-7). The release of [carbonyl-¹⁴C]nicotinamide from [carbonyl-¹⁴C]NAD was determined in the presence or absence of arginine methyl ester for the quantitation of ADP-ribosyltransferase and NAD glycohydrolase activity, respectively. Although high concentrations of potassium phosphate were required for maximal enzymatic activity, there was only 3% nonspecific hydrolysis of [carbonyl-¹⁴C]NAD after a 90-min incubation at 30°C. The concentrations of reagents and specific additions are indicated in the table legends. Adenylate cyclase activity was measured as described (16). Protein concentration was measured by the procedure of Lowry (17).

Preparation of Components of Cholera Toxin—All components of cholera toxin were prepared according to the procedure of Lai *et al.* (18) as described previously (19). A protomer of cholera toxin was well separated from B protomer, and A₁ peptide was also completely resolved from A₂ peptide on a Sephadex G-75 column (1 × 120 cm). Alkylated A₁ peptide appeared as one band in SDS¹-polyacrylamide gel electrophoresis (20).

¹ The abbreviation used is: SDS, sodium dodecyl sulfate.

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Treatment of Liver Plasma Membranes with Cholera Toxin— Partially purified plasma membranes from rat liver were prepared as described previously (21). The mixture for treatment contained, in 100 μ l, 40 mM Tris-Cl, pH 7.5, 1 mM dithiothreitol, 1 mg/ml of bovine serum albumin, plasma membranes (about 1 mg), 2 mM NAD, and cholera toxin or its component. After 5 min at 30°C, the mixture was diluted to 5 ml with 10 mM Tris-Cl, pH 7.5, and centrifuged at 30,000 $\times g$ for 15 min at 0°C. The pellet was resuspended for assay of adenylate cyclase activity.

RESULTS

The purified alkylated A₁ peptide catalyzed the release of [carbonyl-¹⁴C]nicotinamide from [carbonyl-¹⁴C]NAD (Table I). The rate of [carbonyl-¹⁴C]nicotinamide release was enhanced by arginine methyl ester (Table I). In the presence of [adenine-U-¹⁴C]NAD, L-[³H]arginine, and A₁, a product was formed which has properties consistent with those of ADP-ribose-L-arginine (data not shown) (4). No activity was present in the A₂ peptide (Table I); the B subunit was shown previously to be inactive (5). The ratio of [carbonyl-¹⁴C]nicotinamide release in the presence of arginine methyl ester to that observed in its absence was similar for A₁ and cholera toxin (Table I). The turnover numbers for A₁ and cholera toxin were similar (Table I). The NAD glycohydrolase activity and the ADP-ribosyltransferase activity co-chromatographed with A₁ peptide on Sephadex G-75. The specific activities of the peak column fractions in both assays were constant across the protein peak (Table II). The ratio of NAD glycohydrolase activity to ADP-ribosyltransferase activity was also constant (Table II). The fractions which catalyzed the NAD glycohydrolase and ADP-ribosyltransferase reactions also activated adenylate cyclase. The degree of activation was proportional to the amount of A₁ present in the fraction (Table II).

Both the NAD glycohydrolase and ADP-ribosyltransferase activities of A₁ were enhanced by increased potassium phosphate in the assay (Table III) as observed previously with holotoxin (5, 7). In contrast to the holotoxin, however, A₁ was not dependent on dithiothreitol for activity (Table III) nor did it exhibit a lag in activity when the reaction was initiated with toxin (data not shown). The K_m for NAD obtained with A₁ was 5 mM, which is similar to that obtained previously with the holotoxin (data not shown). The ability of the A₁ peptide to activate adenylate cyclase was inhibited by SDS (Table IV). It had been previously shown that SDS inhibited the ADP-ribosyltransferase activity of cholera toxin (23).

To ascertain whether NAD glycohydrolase is associated with the ability of peptides of toxin, prepared in this study, to

TABLE I

Effect of arginine methyl ester on [carbonyl-¹⁴C]nicotinamide release from [carbonyl-¹⁴C]NAD catalyzed by cholera toxin and its purified A₁ and A₂ peptides

Assays contained 400 mM potassium phosphate (pH 7.0), 1 mg/ml of ovalbumin, 2 mM [carbonyl-¹⁴C]NAD (38,200 cpm), 20 mM dithiothreitol, and 75 mM arginine methyl ester where indicated in a total volume of 0.3 ml. The reaction was initiated with holotoxin, A₁, or A₂. After 90 min at 30°C, two 0.1-ml samples were taken for isolation of [carbonyl-¹⁴C]nicotinamide (5, 7). All assays were performed in duplicate.

Additions	[Carbonyl- ¹⁴ C]nicotinamide released	
	-Arginine methyl ester	+Arginine methyl ester
μ g	nmol/min/mg	
Cholera toxin 75	22.4 (1.9) ^a	79.8 (6.7) ^a
A ₁ peptide 13.5	115 (2.7)	438 (10.3)
A ₂ peptide 2.85		3.5 (0.0)

^a Figures in parentheses represent turnover numbers; the molecular weights used for the proteins are: cholera toxin, 84,000; A₁, 23,500; A₂, 5,500 (1, 2, 22).

TABLE II

Co-chromatography of A₁ peptide, NAD glycohydrolase, and ADP-ribosyltransferase activities, and the ability to activate adenylate cyclase

NAD glycohydrolase and ADP-ribosyltransferase assays contained 400 mM potassium phosphate (pH 7.0), 20 mM dithiothreitol, 2 mM [carbonyl-¹⁴C]NAD (46,400 cpm), ovalbumin (1 mg/ml), and 75 mM arginine methyl ester, where indicated, in a total volume of 0.3 ml. NAD glycohydrolase assays were initiated with 10 μ l of the indicated fraction and ADP-ribosyltransferase assays with 2 μ l. After 90 min at 30°C, two 0.1-ml samples were taken for isolation of [carbonyl-¹⁴C]nicotinamide. Adenylate cyclase was assayed as described previously (16) and as noted under "Experimental Procedures." The assay medium consisted of 0.1 mM [α -³²P]ATP, 10 mM MgCl₂, 0.1 mM cyclic AMP, 5 mM creatine phosphate, 0.2 mg/ml of creatine phosphokinase, 10 μ M GTP, 1 mM dithiothreitol, 1 mg/ml of bovine serum albumin, and 40 mM Tris-Cl, pH 7.5.

Fraction no. ^a	Protein	NAD glycohydrolase activity (A)		ADP-ribosyltransferase activity (B)		Ratio A/B	Adenylate cyclase activity ^b
	mg/ml	nmol/assay	nmol/min/mg	nmol/assay	nmol/min/mg		
7	0	3.6		3.6			221
8	0	3.4		0.3			240
9	0.01	1.2		4.5			252
10 ^d	1.17	53.8	51.1	78.2	372	0.14	396
11 ^d	2.75	95.8	38.8	138.0	278	0.14	468
12 ^d	0.89	42.2	52.7	53.0	327	0.16	372
13	0.02	2.0		9.6			276
14	0.01	0		0			256

^a Reduced and alkylated A₁ peptide (~1.2 mg) was chromatographed on a column (1 \times 120 cm) of Sephadex G-75 with 5% formic acid. Fractions (4 ml) were lyophilized. Each fraction was dissolved in 200 μ l of 8 M urea, 1 mM EDTA in 10 mM Tris-Cl, pH 7.5, and renatured by dialysis against decreasing concentrations of urea solution over a 24-h period.

^b Basal adenylate cyclase activity was 236 pmol of cAMP/5 min/mg of liver membrane protein.

^c Concentration in the dialyzed samples.

^d The difference in specific activity in fractions 10, 11, and 12 may result from the procedures necessary to isolate A₁ following chromatography.

TABLE III

Effect of potassium phosphate and dithiothreitol on release of [carbonyl-¹⁴C]nicotinamide from [carbonyl-¹⁴C]NAD catalyzed by A₁

Assays contained 8 mM [carbonyl-¹⁴C]NAD (34,700 cpm) and the indicated additions. Reaction was initiated with A₁ (10 μ g in a final volume of 0.3 ml). After 90 min at 30°C, two 0.1-ml samples were taken for isolation of [carbonyl-¹⁴C]nicotinamide (5, 7).

Additions	[Carbonyl- ¹⁴ C]nicotinamide released	
	No arginine methyl ester	75 mM arginine methyl ester
	nmol/min/mg	
50 mM potassium phosphate (pH 7.0)	13	320
400 mM potassium phosphate	130	980
400 mM potassium phosphate + 20 mM dithiothreitol	140	1000

activate the adenylate cyclase system in liver plasma membranes, the activity of each component was examined. As shown in Table V, both B protomer and A₂ peptide were devoid of detectable activity, whereas intact toxin, A protomer, and A₁ peptide were all capable of activating the adenylate cyclase, and this activation was NAD-dependent. As noted above, the protein which enhances the adenylate cyclase activity did co-chromatograph with the A₁ peptide.

The A₁ peptide was also purified by preparative polyacrylamide gel electrophoresis in 0.1% Triton X-100 and dithio-

TABLE IV

Effect of sodium dodecyl sulfate on the ability of A₁ peptide to activate adenylate cyclase

The A₁ peptide was treated with SDS, at the concentration indicated, for 10 min at 30°C. Then, 15 µl (20 µg of toxin) of this mixture was added to 200 µg of liver membranes in a final volume of 50 µl containing 1.5 mM NAD and 50 mM Tris-Cl, pH 7.5. After 5 min at 30°C, the mixture was diluted with 150 µl of ice H₂O and 40 µl was assayed for adenylate cyclase activity as described. The basal activity (in the absence of toxin) was 236 pmol/mg of protein/5 min. The numbers in parentheses indicate the adenylate cyclase activity if SDS alone is carried into the assay.

SDS	Adenylate cyclase activity ^a
%	
	468
0.003	436 (220)
0.01	424 (228)
0.03	364 (272)
0.1	204 (184)

^a Picomoles/mg of rat liver protein/5 min.

TABLE V

Activation of adenylate cyclase in liver membranes by cholera toxin and its components

The assay for adenylate cyclase was carried out at 30°C for 5 min in a total volume of 100 µl as described previously (16). High concentrations of toxin were used to ensure maximal effect in 5 min of incubation at 30°C. Cholera toxin and the A protomer were treated with 20 mM dithiothreitol for 20 min at 30°C before use. Data are means of values from three assays ± S.E.

Additions	Adenylate cyclase activity
µg	pmol cyclic AMP/mg protein/5 min
None	212 ± 5
Cholera toxin 20	352 ± 8
A protomer 4	420 ± 2
B protomer 10	225 ± 4
A ₁ peptide 10	412 ± 9
A ₂ peptide 5	220 ± 5

TABLE VI

Purification of the A₁ peptide of cholera toxin by preparative polyacrylamide gel electrophoresis

Cholera toxin (400 µg) was incubated with 77 mM dithiothreitol, 0.154% Triton X-100, 38.5 mM Tris-Cl, pH 7.4, 0.8 mM EDTA, 2.3 mM NaN₃, 154 mM NaCl for 2½ h at 2°C. The sample was then mixed with glycerol (final concentration, 10%) and applied to an 8% preparative polyacrylamide gel (1.5/2 × 2 cm) containing 0.1% Triton X-100. Elution buffer: 25 mM Tris-Cl, 0.2 M glycine, pH 8.3, 0.1% Triton X-100, 2 mM dithiothreitol. Reservoir buffer: 25 mM Tris-Cl, 0.2 M glycine, pH 8.3, 0.1% Triton X-100 + 1 mM dithiothreitol (24). Electrophoresis was performed at 15 mA. Fractions (1 ml) were assayed for protein, NAD glycohydrolase, and ADP-ribosyltransferase activities and ability to activate adenylate cyclase in the brain particulate system (25). The first peak off the gel contained all three activities and was further identified as the A₁ peptide: 0.1 ml of each peak fraction (33 to 35) was pooled and proteins precipitated with 5% trichloroacetic acid (4°C). The pellet was dissolved in 1% SDS and applied to a 12% polyacrylamide gel containing 0.1% SDS (see Fig. 1).

Fraction	Protein	NAD glycohydrolase	ADP-ribosyltransferase	Ratio -Arg/+Arg	Adenylate cyclase activity
	µg/ml	nmol/ml	nmol/ml	nmol/min/mg	pmol/mg/min
33	20.3	3.761	185	28.52	1405
34	21.6	4.071	189	31.72	1469
35	20.0	3.778	189	27.78	1389
					(basal 7.4)

threitol (Table VI).² The peptide isolated by this procedure was subsequently examined by polyacrylamide gel electrophoresis in SDS and demonstrated only one protein band with a mobility identical with that of the A₁ peptide of cholera toxin.

² This method permits the resolution of A₁ from the B components without alkylation of the cysteine -SH moiety.



FIG. 1. Co-migration of the protein from preparative polyacrylamide gel electrophoresis with the A₁ peptide of cholera toxin. The protein isolated by preparative polyacrylamide gel electrophoresis (Table VI) was applied to 0.1% SDS, 12% polyacrylamide gels as noted earlier. Left, protein from fractions 33 to 35; right, cholera toxin subjected to dithiothreitol and 0.1% Triton X-100 and trichloroacetic acid precipitation. Upper band on right is A₁; lower band is B peptide.

(Fig. 1). This peptide possessed NAD glycohydrolase and ADP-ribosyltransferase activities and activated adenylate cyclase. The ratio of NAD glycohydrolase to ADP-ribosyltransferase activity was 0.13 (Table VI).

DISCUSSION

In the intact toxin, the A₁ peptide is joined to A₂ through a single disulfide bond (1, 2, 22). The two peptides can be resolved by gel permeation chromatography after alkylation of the -SH groups (18) or by polyacrylamide gel electrophoresis in Triton X-100 and dithiothreitol, as shown in the present report. The latter procedure permits the isolation of an A₁ peptide that has not been covalently modified. Both alkylated and unmodified A₁ catalyzed the activation of adenylate cyclase and possessed NAD glycohydrolase and ADP-ribosyltransferase activities. There was no significant difference in the ratio of NAD glycohydrolase to ADP-ribosyltransferase activity with either modified or unmodified A₁ peptide isolated by chromatography or electrophoresis, respectively. Since alkylated A₁ is as active as holotoxin, it would appear that the cysteine is not critical for enzymatic activity. Ledley *et al.* (26) have noted a sequence homology between the A₁ of cholera toxin and the α subunit of the glycopeptide hormones which involved the cysteine and two adjacent amino acid residues and suggested that this common sequence might imply a similar mechanism of action of both toxin and hormone. Since an intact cysteine is not required for enzymatic activity, however, it is unlikely that the sequence homology between A₁ and α subunit occurs in a critical region and is related to an ability to catalyze similar enzymatic reactions.

As noted previously, the A protomer and intact toxin required high salt (as potassium phosphate, sodium chloride, or acetate) and dithiothreitol in order to demonstrate either glycohydrolase or transferase activity (5, 7). It had been postulated that dithiothreitol was necessary to break the disulfide bridge linking the A₁ and A₂ peptides and that the lag in the assay reflects the time necessary for the action of dithiothreitol. This proposal is supported by the present finding that alkylated A₁ does not show a lag or dithiothreitol requirement. The requirement for high concentrations of potassium phosphate for demonstration of the NAD glycohydrolase activities of toxin and A₁ peptide suggests that high ionic strength is necessary for the intrinsic activity of the toxin and not only to disrupt the subunit structure and release A₁ peptide.

Previous investigators have suggested that SDS enhances the ability of cholera toxin to activate adenylate cyclase (4). Tait and van Heyningen (13) reported that SDS inhibited the NAD glycohydrolase activity of cholera toxin preparations and used this contradiction to suggest that the NAD glycohydrolase activity was not intrinsic to the toxin. We have previously shown that at low toxin concentrations, SDS will stabilize the ADP-ribosyltransferase activity of cholera toxin; it did not, however, activate maximally stimulated toxin (23). As noted in the present report, SDS at high concentrations did inhibit both the enzymatic activity of A₁ as well as its ability to activate adenylate cyclase. The "activation" of cholera toxin noted with SDS by other workers may have resulted either from a stabilization of the toxin at low protein concentrations or from, perhaps, an accelerated release of A₁ peptide from the holotoxin due to the denaturation of the B subunits by the detergent.

The evidence presented in this report is consistent with previous observations that cholera toxin catalyzes an NAD glycohydrolase activity (5, 6). In contrast to the report by Tait and van Heyningen (13) using what appears to be a relatively impure material, all the NAD glycohydrolase activity of this cholera toxin preparation did co-electrophorese with toxin protein and did co-chromatograph with the A protomer (15). All the activity was present in the A₁ peptide of cholera toxin which also catalyzed the ADP-ribosylation of arginine and the NAD-dependent activation of adenylate cyclase. The turnover number for the isolated A₁ peptide in both the glycohydrolase and transferase reactions was similar to that of the parent holotoxin. Thus, cholera toxin is similar to other NAD-dependent bacterial toxins, *Pseudomonas* exotoxin A (14), diphtheria toxin (15), and *Escherichia coli* heat-labile enterotoxin (27), which also possess NAD glycohydrolase in addition to ADP-ribosyltransferase activity.

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REFERENCES

- Gill, D. M. (1977) *Adv. Cyclic Nucleotide Res.* **8**, 85–118
- Van Heyningen, S. (1977) *Biol. Rev.* **52**, 509–549
- Gill, D. M. (1975) *Proc. Natl. Acad. Sci. U. S. A.* **72**, 2064–2068
- Gill, D. M. (1976) *J. Infect. Dis.* **133** (suppl), S55–S63
- Moss, J., Manganiello, V. C., and Vaughan, M. (1976) *Proc. Natl. Acad. Sci. U. S. A.* **73**, 4424–4427
- Moss, J., Osborne, J. C., Jr., Fishman, P. H., Brewer, H. B., Jr., Vaughan, M., and Brady, R. O. (1977) *Proc. Natl. Acad. Sci. U. S. A.* **74**, 74–78
- Moss, J., and Vaughan, M. (1977) *J. Biol. Chem.* **252**, 2455–2457
- Trepel, J. B., Chuang, D. M., and Neff, N. H. (1977) *Proc. Natl. Acad. Sci. U. S. A.* **74**, 5440–5442
- Cassel, D., and Pfeuffer, T. (1978) *Proc. Natl. Acad. Sci. U. S. A.* **75**, 2669–2673
- Gill, D. M., and Meren, R. (1978) *Proc. Natl. Acad. Sci. U. S. A.* **75**, 3050–3054
- Moss, J., and Vaughan, M. (1978) *Proc. Natl. Acad. Sci. U. S. A.* **75**, 3621–3624
- Gill, D. M. (1979) *J. Supramol. Struct.* **10**, 151–163
- Tait, R. M., and Van Heyningen, S. (1978) *Biochem. J.* **174**, 1059–1062
- Kandel, J., Collier, R. J., and Chung, D. W. (1974) *J. Biol. Chem.* **249**, 2088–2097
- Chung, D. W., and Collier, R. J. (1977) *Infect. Immun.* **16**, 832–841
- Salomon, Y., Londos, C., and Rodbell, M. (1974) *Anal. Biochem.* **58**, 541–548
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Lai, C. Y., Mendez, E., and Chang, D. (1976) *J. Infect. Dis.* **133** (suppl), S23–S30
- Lin, M. C., Walton, A. F., and Berman, M. F. (1978) *J. Cyclic Nucleotide Res.* **4**, 159–168
- Fairbanks, G., Steck, T. L., and Wallach, D. F. H. (1971) *Biochemistry* **10**, 2606–2617
- Pohl, S. L., Birnbaumer, L., and Rodbell, M. (1971) *J. Biol. Chem.* **246**, 1849–1856
- Gill, D. M. (1976) *Biochemistry* **15**, 1242–1248
- Moss, J., Ross, P. S., and Vaughan, M. (1978) in *Proceedings of the Thirteenth Joint Conference on Cholera*, Department of Health, Education, and Welfare Publication No. 78-1590, National Institutes of Health, pp. 382–395
- Laemmli, U. K. (1970) *Nature* **227**, 680–685
- Moss, J., and Vaughan, M. (1977) *Proc. Natl. Acad. Sci. U. S. A.* **74**, 4396–4400
- Ledley, F. D., Mullin, B. R., Lee, G., Aloj, S. M., Fishman, P. H., Hunt, L. T., Dayhoff, M. O., and Kohn, L. D. (1976) *Biochem. Biophys. Res. Commun.* **69**, 852–859
- Moss, J., and Richardson, S. H. (1978) *J. Clin. Invest.* **62**, 281–285

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