NADPH complex randomly. It will be interesting to see if the crown gall tumor enzyme exhibits other similarities in its catalytic mechanism to octopine dehydrogenase from *Pecten maximus*.

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Registry No. NADH, 58-68-4; NAD, 53-84-9; L-arginine, 74-79-3; pyruvate, 127-17-3; octopine, 34522-32-2; δ-guanidinovalerate, 462-93-1; propionate, 79-09-4; N^2-ethyl-L-arginine, 88855-11-2; octopine dehydrogenase, 37256-27-2.

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Activation of an Erythrocyte NAD:Arginine ADP-Ribosyltransferase by Lysolecithin and Nonionic and Zwitterionic Detergents†

Joel Moss,* James C. Osborne, Jr., and Sally J. Stanley

ABSTRACT: The activity of an NAD:arginine ADP-ribosyltransferase was stimulated 4–6-fold by lysolecithin; lysolecithins containing long-chain fatty acids such as stearoyl (C_{18}) and palmitoyl (C_{16}) were more effective than those with shorter chains: $C_{14} > C_{12} > C_{10} \simeq C_8$. The analogue lacking a fatty acid at C-1, α-glycerophosphocholine, was inactive as were choline, lysophosphatidic acid, lysophosphatidylserine, lysophosphatidylglycerol, lysophosphatidylethanolamine, lecithin, phosphatidic acid, phosphatidylserine, and phosphatidylethanolamine. Activation of the transferase was, however, also observed with certain nonionic (e.g., Triton X-100) and zwitterionic [3-[(cholamidopropyl)dimethylammonio]-1-propanesulfonate] detergents. The transferase was shown previously to be stimulated by chaotropic salts or histones; in the presence of maximally effective concentrations of lysolecithin, salt, and histone, the activity was similar to that observed in the presence of histone or salt alone. Maximal activation by lysolecithin and detergents was less than that observed with either salt or histone. It appears that activation by lysolecithin shows significant differences from that observed previously with histones or salt and can be mimicked by certain nonionic and zwitterionic detergents.

Covalent modification of proteins plays a critical role in the biological function of many proteins and enzymes. For instance, the transfer of the ADP-ribose moiety of NAD to the regulatory component of adenylate cyclase or to elongation factor II of the protein synthetic pathway appears to be critical to the action of choleragen (cholera toxin) and diphtheria toxin, respectively (Moss & Vaughan, 1979; Pappenheimer, 1977). In the reactions catalyzed by these toxins, it appears that a single ADP-ribose moiety is placed on a critical amino acid. In animal tissues, two distinct types of ADP-ribosyltransferases have been described: one enzyme, poly(ADP-ribose) synthetase, may catalyze both the initial ADP-ribosylation of protein and also the subsequent addition of ADP-ribose moieties to form a polymeric structure (Hayaishi & Ueda, 1977; Pekala & Moss, 1983). A second type, a mono-ADP-ribosyltransferase, catalyzes only the initial ADP-ribosylation of protein; in addition to protein, arginine residues or other compounds containing a guanidino group may serve as ADP-ribose acceptors (Moss & Vaughan, 1978; Moss et al., 1980). An enzyme with this substrate specificity has been purified to apparent homogeneity from turkey erythrocytes; it has a subunit molecular weight of ~28 000 (Moss et al., 1980). This NAD:arginine ADP-ribosyltransferase exists in an inactive aggregated form of high molecular weight and becomes activated upon dissociation. The conversion from the inactive to the active form is promoted by chaotropic salts or histones (Moss et al., 1981, 1982). The activity of the transferase thus appears to be sensitive to the local environment and quaternary structure. Since the enzyme appears to exist

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in both soluble and membrane-bound forms, we investigated whether membrane constituents might alter transferase activity. In the present paper, we demonstrate that the activity of the ADP-ribosyltransferase is specifically stimulated 4–6-fold by lysophosphatidylcholine but not by related phospholipids.

Experimental Procedures

Materials

Egg yolk lysolecithin was obtained from Sigma; unless indicated otherwise, "lysolecithin" refers to this material and source. Before use, the phospholipids were sonicated for 30 s in buffer. Triton X-114 and Triton X-305 were also from Sigma. Soybean lysolecithin and 3-[(cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) were obtained from Calbiochem; choline was from Matheson Coleman and Bell; [*carbonyl*-^{14}C]NAD (53 mCi/mmol) and [*adenine*-U-^{14}C]NAD (286 mCi/mmol) were from Amersham; lauroyl-, myristoyl-, palmitoyl-, and stearoyllysolecithin, NAD, histone, arginine methyl ester, agmatine sulfate, argininic acid, arginine, and guanidinopropionate were from Sigma; capryl- and caprylyllysolecithin were from P-L Biochemicals; dithiothreitol was from Bethesda Research Laboratories; guanidine was from Schwarz/Mann; Tween 20 was from Fisher; and Triton X-100 was from Research Products International.

Methods

Assays. ADP-ribosyltransferase and NAD glycohydrolase activities were determined as described previously (Moss & Vaughan, 1977; Moss et al., 1976). The standard assay contained 50 mM potassium phosphate, pH 7.0, 1 mg/mL ovalbumin, 32.4 μM [*carbonyl*-^{14}C]NAD (~40000 cpm), and the indicated additions in a total volume of 0.3 mL. ADP-ribosyltransferase assays contained 2 mM agmatine; this concentration was chosen in part to optimize the apparent activation by phospholipid, chaotropic salt (Moss & Stanley, 1981; Moss et al., 1981), and histone (Moss & Stanley, 1981; Moss et al., 1982). The reaction was initiated with erythrocyte ADP-ribosyltransferase (~1.21 ng). After incubation for 30 min at 37 °C, or as indicated, two 0.1-mL samples were fractionated on AG 1-X2 columns to isolate [*carbonyl*-^{14}C]-nicotinamide (Moss et al., 1976). To directly measure ADP-ribose–agmatine formation, [*adenine*-U-^{14}C]NAD was used in the assay; [*adenine*-U-^{14}C]ADP-ribose–agmatine was isolated by AG 1-X2 chromatography (Moss & Stanley, 1981). All assays were performed in duplicate. Protein was determined by the method of Lowry et al. (1951) using bovine serum albumin as standard.

Enzyme Purification. The ADP-ribosyltransferase was purified from turkey erythrocytes as described previously (Moss et al., 1980). The protein exhibited one major band by sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

Preparation of Plasma Lipoproteins. Very low density lipoproteins (d < 1.006 g/mL), low-density lipoproteins (1.006–1.063 g/mL), and high-density lipoproteins (1.063–1.21 g/mL) were isolated by sequential ultracentrifugation in a Beckman 60 Ti rotor (Beckman Inc., Fullerton, CA) (Havel et al., 1955).

Results

The activity of the erythrocyte NAD:arginine ADP-ribosyltransferase was enhanced ~6-fold by phospholipids (Table I); both animal (egg yolk) and plant (soybean) lysolecithins were highly effective (data not shown) as were lysophosphatidylcholine derivatives containing palmitoyl (C_{16}) > stearoyl (C_{18}) > C_{14} > C_{12} > C_{10} ≃ C_8 side chains (Figure 1); lysophosphatidylglycerol, lysophosphatidylserine, lysophosphatidylethanolamine, and lysophosphatidic acid did not increase transferase activity; glycerophosphatidylcholine and choline (data not shown) were inactive. No effect was observed with phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylglycerol, and phosphatidic acid (Table I).

Table I: Effect of Phospholipids on the Activity of Erythrocyte ADP-Ribosyltransferase

additions (0.3 mg/mL)	[*carbonyl*-^{14}C]-nicotinamide released (pmol·min^{-1})
none	8.8
lysophosphatidylcholine	41.1
phosphatidylcholine	9.0
phosphatidylserine	8.9
phosphatidylinositol	7.1
none	6.0
lysophosphatidylcholine	57.5
phosphatidylethanolamine	8.4
phosphatidylglycerol	6.4
phosphatidic acid (palmitoyl)	6.3
lysophosphatidic acid (palmitoyl)[a]	2.9
lysophosphatidylethanolamine (0.1 mg/mL)	8.2
none	7.3
lysophosphatidylcholine	32.2
lysophosphatidylserine	9.1
lysophosphatidylglycerol	3.6
glycerophosphocholine	11.3

[a] Compound obtained from Serdary Research Laboratories; all others were from Sigma. The standard assay prepared with the indicated additions was initiated with ADP-ribosyltransferase, as noted under Experimental Procedures.

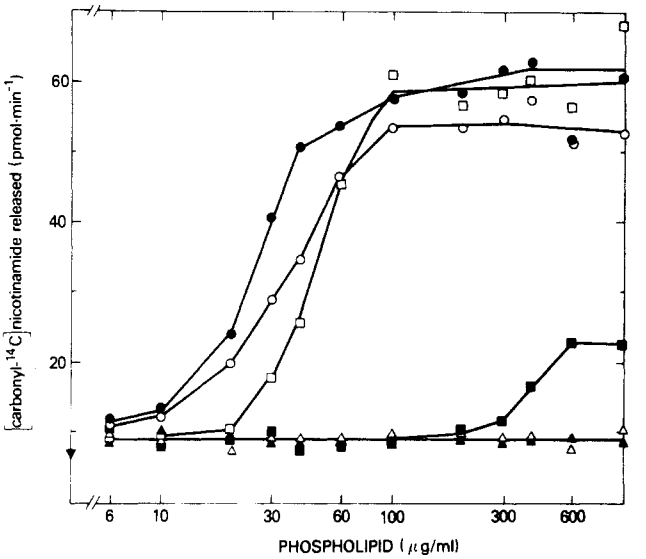

FIGURE 1: Effect of lysophosphatidylcholine-containing fatty acids of different chain lengths on the activity of erythrocyte ADP-ribosyltransferase. Reaction mixes were prepared as described under Experimental Procedures with the following indicated additions: stearoyllysophosphatidylcholine (○); palmitoyllysophosphatidylcholine (●); myristoyllysophosphatidylcholine (□); lauroyllysophosphatidylcholine (■); capryllysophosphatidylcholine (△); caprylyllysophosphatidylcholine (▲); reaction was initiated with erythrocyte ADP-ribosyltransferase (1.21 ng) and run as described under Experimental Procedures.

Activation of the ADP-ribosyltransferase by lysolecithin was rapid (Figure 2). The lack of transferase activity in the absence of lysolecithin was not due to inactivation of the enzyme; addition of lysolecithin to enzyme previously incubated in lysolecithin-free assay mix led to a rapid increase in activity

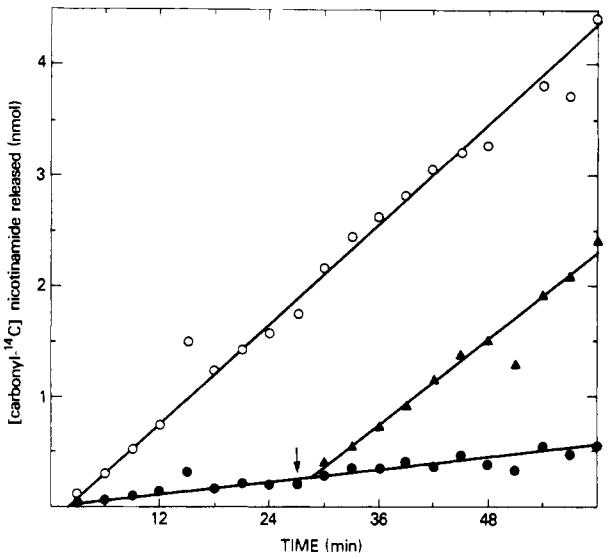

FIGURE 2: Time course for the effect of lysolecithin on the activity of NAD:arginine ADP-ribosyltransferase. ADP-ribosyltransferase (24.2 ng) was added to a standard reaction mix (4.5 mL) with (○) or without (●) lysolecithin (0.3 mg/mL). At 27 min, lysolecithin (45 μL) was added to a reaction mix (4.5 mL) initiated at $t = 0$ with transferase (▲). Two 0.1-mL samples were withdrawn at the indicated times to assess activity.

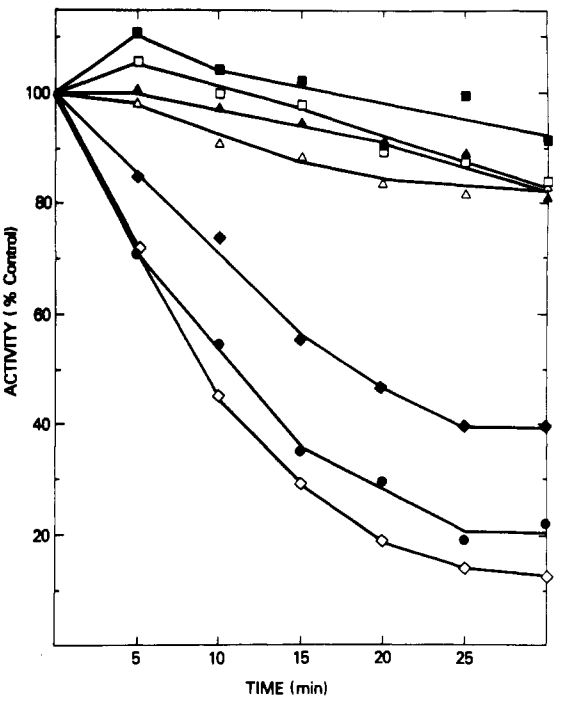

FIGURE 3: Effect of lysolecithins containing fatty acids of different chain lengths on the stability of ADP-ribosyltransferase. ADP-ribosyltransferases (24.2 ng) were incubated in duplicate at 30 °C for the indicated times in the absence (●) of lysolecithin or in the presence of 0.3 mg/mL egg lysolecithin (▲) or stearoyl- (△), palmitoyl- (■), myristoyl- (□), lauroyl- (◆), or (◇) capryllysolecithin in a final volume of 0.3 mL. Two 10-μL samples were then assayed for 30 min at 30 °C in a mix containing 50 mM potassium phosphate, pH 7.0, ovalbumin (1 mg/mL), 32.4 μM [*carbonyl*-^{14}C]NAD (33 400 cpm), 6 mM agmatine, and 300 mM NaCl.

(Figure 2). Activation by phospholipid was also reversible; dilution of lysolecithin-activated enzyme into lysolecithin-free assay mix resulted in a loss in transferase activity (data not shown). In addition to its effects on transferase activity, lysolecithin also stabilized the enzyme against thermal denaturation (Figure 3); enzyme inactivated in the absence of phospholipid was not reactivated by the addition of lysolecithin (data not shown). Those lysolecithins (C_{18}, C_{16}, C_{14}) that were effective activators of the enzyme also stabilized; C_{12} was clearly less active while C_{10} was inactive. Activation was not necessarily associated with stabilization, since 300 mM NaCl, which enhances enzyme activity, did not prevent thermal inactivation.

Lysolecithin increased the ADP-ribosylation of both low molecular weight guanidino compounds and model protein acceptors (data not shown). The ability of lysolecithin to cause a stimulation of protein ADP-ribosylation was highly selective for the proteins tested. In contrast, it appeared to stimulate the ADP-ribosylation of all the low molecular weight guanidino compounds.

It was noted previously that the activity of the erythrocyte transferase was enhanced by chaotropic salts or histones (Moss & Stanley, 1981; Moss et al., 1981, 1982). Lysolecithin was clearly not as effective as either of these agents in activating the transferase (Table II); lysolecithin did not further stimulate transferase assayed at maximally effective salt or histone concentrations (Table II).

Assays performed with either [*carbonyl*-^{14}C]NAD or [*adenine*-U-^{14}C]NAD to monitor [*carbonyl*-^{14}C]nicotinamide release or to determine directly [*adenine*-U-^{14}C]ADP-ribose–agmatine formation, respectively, gave similar activation by lysolecithin (Table II). The ratio of [*adenine*-U-^{14}C]ADP-ribose coupled to agmatine to [*carbonyl*-^{14}C]nicotinamide release was ~0.85. Thus, lysolecithin did not appear to preferentially stimulate NAD hydrolysis.

The apparent K_m for NAD in lysolecithins was 14 μM, whereas it was 25 μM in assays containing NaCl. The V_{max} in NaCl was, however, greater (~1.7-fold) than that in lysolecithin (data not shown). The presence of NaCl in assays containing lysolecithin resulted in an increase in the V_{max} and the apparent K_m when compared to those seen with lysolecithin alone. In contrast to the apparent K_m for NAD, which varied with activator, the apparent K_m for agmatine (~1 mM) was not significantly different in NaCl, lysolecithin, or lysolecithin + NaCl. The V_{max} in lysolecithin was less than that observed in NaCl while the V_{max} in lysolecithin + NaCl was only slightly less than that observed in NaCl alone (data not shown).

Table II: Effect of Lysolecithin, NaCl, Histone, and CHAPS on the Activity of an Erythrocyte ADP-Ribosyltransferase[a]

additions	[*carbonyl*-^{14}C]-nicotinamide released, *A* (pmol·min^{-1})	[*adenine*-U-^{14}C]ADP-ribose incorporated, *B* (pmol·min^{-1})	*B*/*A* ratio
none	13.8	10.3	0.75
lysolecithin (0.3 mg/mL)	81	69	0.85
NaCl (300 mM)	135	117	0.84
NaCl + lysolecithin	140	132	0.94
histone (100 μg/mL)	133	116	0.87
histone + lysolecithin	136	122	0.89
CHAPS (3%)	45	41	0.84
CHAPS + lysolecithin	46	38	0.84

[a] Reaction was initiated in a standard assay mix containing ovalbumin (1 mg/mL). Data given are for egg yolk lysolecithin (0.3 mg/mL); similar results were obtained with oleoyl-, palmitoyl-, stearoyl-, and myristoyllysolecithin at 0.3 mg/mL.

Detergents were examined for their ability to increase ADP-ribosylation. The zwitterionic detergent CHAPS and certain nonionic detergents (e.g., Triton X-100, Triton X-114, Tween 20, and Triton X-305) enhanced enzymatic activity (Table II, Figure 4).

Activation of transferase by CHAPS was also associated with an increase in ADP-ribosylation of agmatine coupled to the release of [*carbonyl*-^{14}C]nicotinamide. In some cases

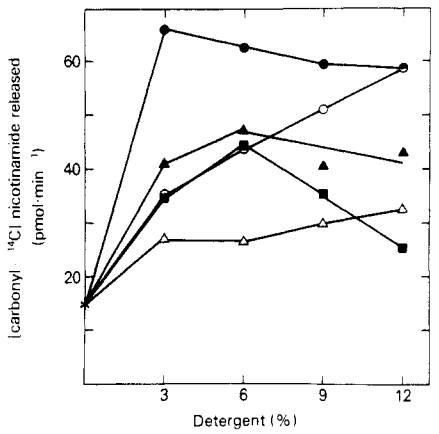

FIGURE 4: Effect of detergents on the release of [*carbonyl*-^{14}C]-nicotinamide from [*carbonyl*-^{14}C]NAD catalyzed by erythrocyte ADP-ribosyltransferase. ADP-ribosyltransferase (1.21 ng) was assayed in an assay mix containing 50 mM potassium phosphate (pH 7.0), 32.4 μM [*carbonyl*-^{14}C]NAD, 6 mM agmatine, 1 mg/mL ovalbumin, and the following indicated detergents: CHAPS (●); Triton X-100 (○); Triton X-114 (▲); Triton X-305 (■); Tween 20 (△).

(Triton X-305), activation was associated with a clearly defined optimal detergent concentration. As previously noted with lysolecithin, the detergents also stabilized the transferase against thermal denaturation (data not shown). Maximal activation by CHAPS was less than that obtained with lysolecithin, histone, or NaCl (data not shown). In the presence of CHAPS and lysolecithin, activity was only slightly less than that obtained with NaCl or histone alone. Physiological micelles such as low-density lipoprotein, very low density lipoprotein, and high-density lipoprotein did not activate the transferase.

Discussion

The present results show that certain lysophosphatidylcholines, but not related phospholipids, activate an NAD:arginine ADP-ribosyltransferase. It appears that both the fatty acid and choline moieties are critical to the activation. Stearoyl- and palmitoyllysophosphatidylcholine were more effective than lysolecithins containing shorter chain fatty acids with myristoyl (C_{14}) > lauroyl (C_{12}) > capryl (C_{10}) = caprylic (C_8); the latter two derivatives were essentially inactive, as were the fatty acid free compounds α-glycerophosphatidylcholine and choline. The importance of the choline moiety is illustrated by the fact that lysophosphatidylethanolamine, lysophosphatidic acid, lysophosphatidylserine, and lysophosphatidylglycerol were inactive.

Activation of transferase by lysolecithin occurs at concentrations of phospholipid that are at or above the critical micelle concentration (Hayaishi et al., 1973). The increase in activity as a function of increasing concentrations of lysolecithin (Figure 1) is highly cooperative and is characteristic of processes involving micelle formation. The binding of apolipoprotein A-II to lysolecithin, which is believed to involve protein–micelle interaction, is also highly cooperative and occurs over the same lysolecithin concentration range as the observed transferase activation (Palumbo & Edelhoch, 1977). The ability of lysolecithin micelles to activate the transferase was mimicked by certain nonionic and zwitterionic detergents; not all agents capable of forming micelles were able to activate the enzyme.

CHAPS, a zwitterionic detergent, appeared to be the most effective agent in terms of the extent of activation and in having a broad plateau at maximal activation prior to the onset of significant inhibition. Since its synthesis by Hjelmeland (1980), this detergent has proven to be extremely useful in the solubilization and purification of many membrane proteins. These studies demonstrate that the detergent affects the activity of soluble as well as membrane proteins and has properties not present in nonionic detergents.

In addition to activating the enzyme, lysolecithin and nonionic and zwitterionic detergents also enhanced its stability. NaCl, which also activates the transferase, did not confer a similar degree of stabilization. Thus, activation and stabilization are not necessarily coupled. Ovalbumin, previously shown to partially stabilize the enzyme, did not activate the transferase (Moss et al., 1979).

The structural requirements in the phospholipid in order to observe activation of the transferase may reflect those features necessary to form an effective interface for stabilization of the active conformation of the transferase. A direct effect of lysolecithin on the transferase is supported by the finding that, in the absence of protein (except 1 ng of enzyme) and low molecular weight guanidino compounds, lecithin stimulated the transferase-catalyzed hydrolysis of NAD to ADP-ribose and nicotinamide. Using structurally simple model substrates such as guanidine, a major effect of lysolecithin was observed on ADP-ribosyltransferase activity; significant activation was obtained with all the low molecular weight guanidino compounds examined. The changes in V_{max} observed with the addition of salt to assays containing lysolecithin are consistent with a direct interaction between the transferase and lysolecithin. The observed increase in stability in the presence of lysolecithin also supports direct binding of the transferase to lysolecithin micelles. Similar increases in stability due to protein–micelle complexes have been observed for apolipoprotein A-II (Palumbo & Edelhoch, 1977). Direct micellar activation of the transferase is supported further by the observation that the apparent K_m for agmatine is not changed in the presence of lysolecithin.

Previous studies demonstrated that the NAD:arginine ADP-ribosyltransferase was activated by chaotropic salts or histones (Moss & Stanley, 1981; Moss et al., 1981, 1982). Both agents increased activity to the same extent; maximal activity observed in the presence of both histones and salt appeared to be identical with that observed with optimal concentrations of either agent alone. Transferase activity in lysolecithin and detergents was clearly less than that obtained with salt or histones; at saturating concentrations of salt or histone, lysolecithin had a slight effect on activity.

Although the activation of NAD:arginine ADP-ribosyltransferase by lysolecithin is abolished by high concentrations of NaCl, on the basis of in vitro studies, it should only be partially activated in vivo at physiological salt concentrations. Thus, there may be further effects on activity resulting from the production of lysophosphatidylcholine. Generation of lysophosphatidylcholine by the action of phospholipase A_2 may enhance the ADP-ribosylation of critical arginine residues in proteins. Since phospholipase A_2 activity is increased by hormones such as bradykinin, conceivably these agents could exert their effects on cells in part through an increase in ADP-ribosylation.

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Registry No. NAD, 53-84-9; CHAPS, 75621-03-3; Triton X-100, 9002-93-1; Triton X-114, 9036-19-5; Triton X-305, 9002-93-1; Tween 20, 9005-64-5; stearoyllysophosphatidylcholine, 19420-57-6; palmitoyllysophosphatidylcholine, 17364-16-8; myristoyllyso-

phosphatidylcholine, 20559-16-4; lauroyllysophosphatidylcholine, 20559-18-6; capryllysophosphatidylcholine, 22248-63-1; caprylyllysophosphatidylcholine, 45287-18-1; NAD:arginine ADP-ribosyltransferase, 81457-93-4; agmatine, 306-60-5.

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Near- and Far-Ultraviolet Circular Dichroism of the Catalytic Subunit of Adenosine Cyclic 5′-Monophosphate Dependent Protein Kinase†

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ABSTRACT: The circular dichroism spectrum of the catalytic subunit of cAMP-dependent protein kinase was measured in the far-UV (190–240 nm) and near-UV (250–300 nm) region. Data from the far-UV spectra were processed with the CONTIN program for estimation of globular protein secondary structure [Provencher, S. W. (1982) *CONTIN (Version 2) User's Manual*, European Molecular Biology Laboratory, Heidelberg, West Germany]. The composition of the protein determined by this method was 49 ± 2% α-helix, 20 ± 4% β-sheet, and 31 ± 3% remainder. This composition changes when the protein is allowed to bind Kemptide, a synthetic peptide substrate, with more than half of the disordered portion of the protein taking the form of β-sheet. A certain portion of the α-helical structure also appears to move into a β-sheet form. The near-UV CD spectrum of catalytic subunit shows changes in aromatic amino acid dichroism associated with substrate binding. These changes can be ascribed with a fair degree of certainty to alterations in the orientation of a tyrosine residue at the surface of the protein. These findings are discussed in terms of previous work on induced dichroism in this enzyme with regard to control mechanisms operating at the active site.

The catalytic subunit of cAMP-dependent protein kinase (EC 2.7.1.37) phosphorylates serine or, less often, threonine residues on substrate proteins. The inactive holoenzyme is a tetramer of two regulatory and two catalytic subunits; the catalytic subunit is freed in active form on the binding of two cAMP molecules per regulatory subunit (Langan, 1967). The enzyme thus acts as the target for cAMP-mediated hormonal responses and might be expected to exhibit a high degree of specificity in its selection of protein substrate.

In isolated form in vitro, however, the catalytic subunit appears to phosphorylate any protein having an accessible serine or threonine in the specific primary sequence Arg-Arg-X-Ser (Daile et al., 1975; Kemp et al., 1977; Kemp, 1978; Feramisco et al., 1979; Meggio et al., 1981). A search for further control points is indicated. This search can be directed both toward looking for external controls such as the family of small, acid-stable proteins and toward understanding the normal function of this enzyme to show at what stages control factors might operate. With this latter goal in mind, we have recently used techniques of induced circular dichroism to observe specific conformational changes at the active site of the enzyme. Initial studies have shown that the binding of protein substrate induces a conformational change at the ATP-binding site that occurs in at least two discrete parts, one dependent on the basic subsite characteristic of the specific primary structure required and one dependent on the presence of a hydroxyl group on the target serine (Reed & Kinzel, 1984). There was some indication that this latter movement was triggered by interaction between the hydroxyl group and a tyrosine residue—one of which is known to be present at the surface of the ATP binding site (Witt & Roskoski, 1975). In an attempt to clarify this interaction, we decided to examine the ultraviolet circular dichroism (UV CD) spectrum of protein kinase catalytic subunit for any changes in intrinsic dichroism, especially that associated with tyrosine, which might be con-

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