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DUAL INHIBITORY EFFECTS OF DIMETHYL SULFOXIDE ON POLY(ADP-RIBOSE) SYNTHETASE

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Dimethyl sulfoxide (DMSO), a solvent popularly used for dissolving water-insoluble compounds, is a weak inhibitor of poly(ADP-ribose) synthetase, that is a nuclear enzyme producing $(ADP\text{-ribose})_n$ from NAD^+. The inhibitory mode and potency depend on the concentration of substrate, NAD^+, as well as the temperature of the reaction; at micromolar concentrations of NAD^+, the inhibition by DMSO is biphasic at 37°C, but is monophasic and apparently competitive with NAD^+ at 25°C. DMSO, on the other hand, diminishes dose-dependently and markedly the inhibitory potency of benzamide and other inhibitors. Other organic solvents, ethanol and methanol, also show a biphasic effect on the synthetase activity at different concentrations.

Keywords: Poly(ADP-ribose) polymerase; ADP-ribosyltransferase; NAD^+; Benzamide; 1,5-Dihydroxyisoquinoline; 6(5*H*)-Phenanthridinone

Abbreviations: DMSO, dimethyl sulfoxide; ADH, alcohol dehydrogenase; ACh, acetylcholine; AChE, acetylcholinesterase; IC_{50}, the concentration to cause 50% inhibition

INTRODUCTION

Poly(ADP-ribose) synthetase [also termed poly(ADP-ribose) polymerase] [NAD^+:poly(adenosine-diphosphate-D-ribosyl)-acceptor ADP-D-ribosyltransferase] (EC 2.4.2.30) is a nuclear enzyme that catalyzes a transfer of

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the ADP-ribose moiety of NAD^+ to an acceptor protein (initiation), and then to this protein-bound ADP-ribose (elongation) to form a chain of $(ADP\text{-ribose})_n$ [poly(ADP-ribose)] with occasional branching.[1-5] The enzymatic activity is unique in its absolute dependency on the presence of DNA with strand termini. Various nuclear proteins, including histones, nonhistone proteins, and the enzyme itself (automodification), serve as acceptors of poly(ADP-ribose) *in vitro* as well as *in vivo*.[2-5] Biological roles suggested for poly(ADP-ribose) or poly(ADP-ribosyl)ation of proteins include implications in DNA repair,[6,7] cell differentiation,[8-11] cell cycle control,[12,13] transformation,[14,15] transcription,[16,17] alteration of chromatin architecture,[18-20] and apoptotic (or non-apoptotic) cell death of neurons[21-28] and other cells.[29-34] However, more recent data obtained with the synthetase gene-knocked out mice show that this enzyme may not be indispensable for DNA repair and apoptosis.[35,36]

Inhibitors of poly(ADP-ribose) synthetase have played a critical role in these studies on biological functions. However, most of previously known inhibitors are accompanied by various side actions *in vivo*,[2,5,37] and their use has left more or less inconclusiveness to interpretation of the results. Therefore, we made a large-scale survey of more than 500 compounds for the inhibitory activity, and found a number of specific and potent inhibitors with a variety of chemical structures.[11,38-43] One technical difficulty in dealing with many of these inhibitors is their low solubilities in the aqueous milieu. We have overcome this difficulty by dissolving them in dimethyl sulfoxide (DMSO) and mixing the solution into the reaction mixture. DMSO dissolves practically all compounds of interest to us and is completely miscible with water. The advantage in the use of this organic solvent as a sole vehicle is that we can divide all compounds into only two groups, water-soluble and DMSO-soluble, which makes a comparison easier. The disadvantage in the use of DMSO is that it has its own inhibitory, though weak, effect on poly(ADP-ribose) synthetase.[39,40,42,44] In this paper, we report in more details *in vitro* effects of DMSO on poly(ADP-ribose) synthetase in the light of interaction with other inhibitors. Effects of DMSO on another ADP-ribosyltransferase, arginine-specific mono(ADP-ribosyl)transferase A (EC 2.4.2.31), are also discussed briefly.

MATERIALS AND METHODS

Enzyme preparations, assays, and kinetic studies of poly(ADP-ribose) synthetase were as described previously.[39,40] Briefly, the standard assay mixture

contained 200 μM [adenosine-^{14}C]NAD^+ (2680 cpm/nmol), 33 μg/ml DNA (calf thymus), 10 mM $MgCl_2$, 5 mM dithiothreitol, and 100 mM Tris/HCl (pH 8.0). The mixture was incubated for 10 min at 37°C, and, after the addition of ice-cold 20% trichloroacetic acid, protein-bound [^{14}C](ADP-ribose)$_n$ was collected on a Millipore filter, and the acid-insoluble ^{14}C was quantified with liquid scintillation. Mono(ADP-ribosyl)transferase A, purified from turkey erythrocytes,[45] was a kind gift of Dr. J. Moss (NIH, USA). The assay of mono(ADP-ribosyl)transferase A was performed by incubating the mixture containing 100 μM [adenosine-^{14}C]NAD^+ (12750 cpm/nmol), 250 μg/ml histone f_{2b} (mainly H2B, calf thymus, Sigma: type VII-S, H-4255), and 50 mM potassium phosphate (pH 7.0) with enzyme for 30 min at 30°C, and quantifying ^{14}C incorporated into acid-insoluble material as described above. The pH of buffers was adjusted in 1 M stock solutions at 20°C.

NAD^+, DMSO, and other chemicals were of the best quality commercially available, as described earlier.[40]

RESULTS

Inhibition of poly(ADP-ribose) synthetase by DMSO depended on the concentration of substrate, NAD^+ (Figure 1). DMSO at concentrations of 0.2–10% (v/v; *ca.* 28–1400 mM) inhibited weakly the enzyme activity under the standard conditions of 200 μM NAD^+, and more strongly at 1 and

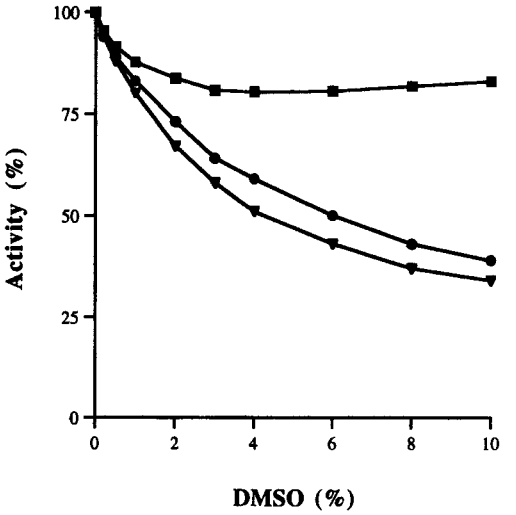

FIGURE 1 Inhibition of poly(ADP-ribose) synthetase activity by DMSO. The reaction was carried out at 37°C and 200 μM (■), 1.0 μM (●), or 0.1 μM (▼) NAD^+.

0.1 μM NAD^+. The IC_{50} values of DMSO in the latter conditions were 6% and 4%, respectively.

The inhibition by DMSO, under the standard conditions, did not show a simple dependency on concentrations, but rather a complex, almost biphasic, effect (Figure 2). The enzyme activity was weakly (up to *ca.* 20%) inhibited by low concentrations (around 5%) of DMSO, partly recovered at intermediate concentrations (10–25%), and strongly inhibited at higher concentrations (> 30%). At the concentration of 50%, the inhibition by DMSO was 98%. Other organic solvents, ethanol and methanol, also showed a biphasic effect on the synthetase activity. Ethanol and methanol showed maximal activation of 212% and 182% at 15% and 20%, respectively, whereas both solvents inhibited markedly the synthetase activity at 30% or higher concentrations. This result is in general agreement with a previous report of marked stimulation (337%) of poly(ADP-ribose) synthesis by 7% ethanol and inhibition at concentrations above 15% in permeabilized L cells.[46]

The inhibitory effect of DMSO was significantly stronger at lower temperatures. At 25°C, for example, 2%, 10%, and 20% DMSO caused 22%, 39%, and 55% inhibition, respectively; the concentration dependency was monophasic in this condition (Figure 2).

Kinetic analysis, carried out by assays at 25°C for 30 s, revealed that DMSO is a competitive inhibitor of poly(ADP-ribose) synthetase with

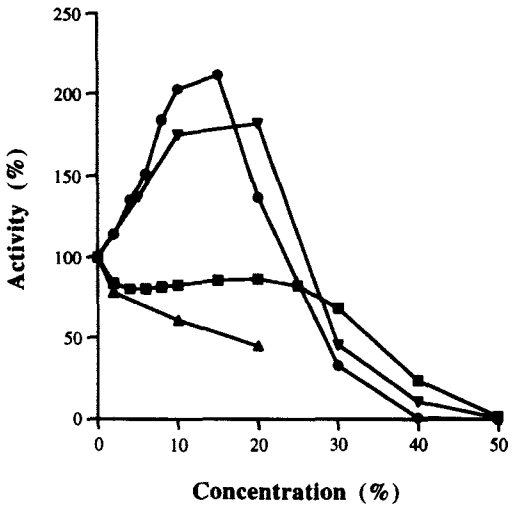

FIGURE 2 Effects of DMSO (■, ▲), ethanol (●), and methanol (▼) on the activity of poly(ADP-ribose) synthetase. The reaction was carried out at 37°C for 10 min (■, ●, ▼) or at 25°C for 30 s (▲).

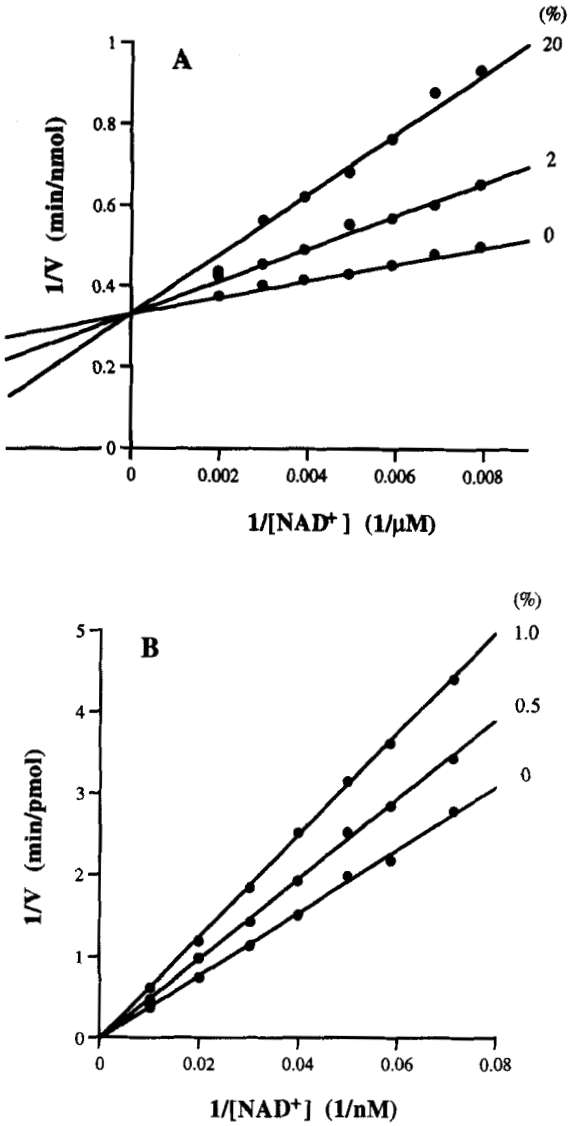

FIGURE 3 Mode of inhibition of poly(ADP-ribose) synthetase activity by DMSO at (A) micromolar or (B) nanomolar concentrations of NAD^+. The reactions were carried out at 25°C for 30 s.

respect to NAD^+ at micromolar concentrations (126–501 μM; cf. K_m = 61 μM) (Figure 3A). DMSO inhibited the enzyme activity also at nanomolar concentrations (14–100 nM) of NAD^+ (Figure 3B), indicating the action at the initiation step of poly(ADP-ribose) synthesis.[47] Under these

conditions, the poly(ADP-ribose) synthesis obeyed first-order kinetics, thereby Lineweaver–Burk or any other plot failed to give information about the mode of action of DMSO.[40]

The presence of DMSO diminished effects of all other inhibitors tested on poly(ADP-ribose) synthetase (Table I). The diminishing effect was remarkable under certain conditions; for example, 1 mM 1,4-benzoquinone exhibited 78% inhibition in a purely aqueous medium, but only 2% inhibition in the presence of 10% DMSO. Furthermore, the effect of DMSO was dose-dependent; for example, the IC_{50} value of 6(5*H*)-phenanthridinone was 0.16 μM in the presence of 0.2% DMSO, but was 0.3 μM or 5.2 μM at 2% or 10% of DMSO, respectively. IC_{50} values of water-soluble inhibitors also differed approximately 2- and 10-fold in the milieu containing 2% or 10% DMSO, respectively (Table I). Thus, a comparison of various inhibitors is feasible only when they are tested at a constant and minimal concentration of DMSO and a fixed concentration of NAD^+.

TABLE I Effect of DMSO on IC_{50} values of various inhibitors of poly(ADP-ribose) synthetase

Inhibitor[a]	*IC_{50} (μM) at DMSO*[b]			
	0%	0.2%	2%	10%
DMSO-soluble				
4-Amino-1,8-naphthalimide	—[c]	—	0.18	0.87
6(5*H*)-Phenanthridinone	—	0.16	0.30	5.2
1,8-Naphthalimide	—	—	1.4	4.4
Luminol	—	—	23	130
Phthalhydrazide	—	—	30	150
m-Phthalamide	—	23	50	210
3-Bromobenzamide	—	—	55	280
2-Fluorobenzamide	—	—	120	360
5-Chlorosalicylamide	—	105	190	—
Xanthurenic acid	—	—	190	800
4-Fluorobenzamide	—	—	200	940
α-Tetralone	—	—	310	1400
5-Nitrouracil	—	210	430	950
6-Aminocoumarin	—	—	850	3300
Water-soluble				
3-Fluorobenzamide	20	—	—	230
3-Chlorobenzamide	22	—	—	220
Benzamide	22	30	55	200
3-Nitrobenzamide	160	—	—	1200
Nicotinamide	210	—	400	—
4-Chlorobenzamide	300	—	—	2900
1,4-Benzoquinone	400	—	—	5000
Cyclohexanecarboxamide	620	—	—	3300

[a] DMSO- and water-soluble inhibitors are arranged in order of the IC_{50} values at 2% or 0% DMSO, respectively. [b] Controls contain the same concentrations of DMSO. [c] Not determined.

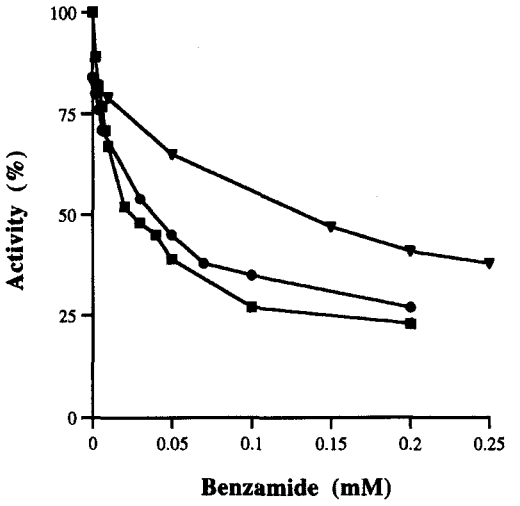

FIGURE 4 Effect of benzamide on the activity of poly(ADP-ribose) synthetase in the absence (■) or the presence of 2% (●) or 10% (▼) DMSO. The reaction was carried out at 37°C for 10 min.

Figure 4 shows changes in inhibition by increasing concentrations of benzamide, a well-known inhibitor, in the absence and presence of 2% or 10% DMSO. IC_{50} values calculated from this plot for benzamide were 37 and 127 μM in 2% and 10% DMSO, respectively. These values are lower than those given in Table I; the controls in this case were without DMSO. IC_{50} values thus calculated might be more useful for estimation of *in vivo* effects than those based on DMSO-matched controls, because the former values reflect the real degrees of inhibition in the presence of DMSO. Although inhibition by benzamide was reduced by DMSO at benzamide concentrations higher than 8–10 μM, the inhibition was rather enhanced at lower concentrations of benzamide by DMSO. This indicates that the interaction of DMSO and other inhibitors with poly(ADP-ribose) synthetase is not simply a competition for binding at the same site(s) on the enzyme molecule.

Effects of DMSO on various inhibitors of other ADP-ribosyltransferases remain to be investigated. A preliminary analysis indicated that IC_{50} values of several inhibitors of arginine-specific mono(ADP-ribosyl)transferase A from turkey erythrocytes also depend on the concentrations of NAD^+ as well as DMSO (Table II).[42] Again, a comparison of their effects on this ADP-ribosyltransferase could be done only by using specified concentrations of NAD^+ and DMSO.

TABLE II Effect of NAD^+ concentration on IC_{50} values of various inhibitors of mono(ADP-ribosyl)transferase A

Inhibitor	*IC_{50} (μM) at NAD^+ concentration of*		
	30	100	200
DMSO-soluble[a]			
6(5*H*)-Phenanthridinone[b]	16	47	—[c]
6(5*H*)-Phenanthridinone[d]	—	77	—
Water-soluble			
1,5-Dihydroxyisoquinoline	37	130	250
Novobiocin	290	370	390
4-Hydroxyquinazoline	320	1000	—
1-Hydroxyisoquinoline	520	1400	—

[a] Controls contain the same concentrations of DMSO. [b] In 2% DMSO. [c] Not determined. [d] In 10% DMSO.

DISCUSSION

DMSO has been shown to affect activities of many enzymes *in vitro*, and a variety of biological actions have been assigned to DMSO (for review, see Ref. [48–51]). In most of these cases, the molecular bases of the effects have not been well understood, and this lack of knowledge hinders its wider use in *in vivo* experiments.

DMSO is known to inhibit horse liver alcohol dehydrogenase (ADH or LADH),[52] vertebrate[53,54] or molluscan[53] acetylcholinesterase (AChE), and cytochrome P-450 isozyme LM3a.[55] The K_i value of DMSO reported for LADH is 5 mM.[52] The inhibition is of a competitive type, and DMSO is assumed to compete with the substrate, butyrylaldehyde, for binding at the carbonyl group. The carbonyl binding site harbors a zinc atom, and DMSO is known to form a complex with zinc at its oxygen atom,[56] suggesting that DMSO reacts with the zinc atom to form an LADH-NADH-DMSO ternary complex. Poly(ADP-ribose) synthetase is another zinc-containing enzyme;[57] it possesses two zinc atoms in a pair of finger structures in the DNA binding domain, and both zinc atoms are necessary for the activity.[58] We previously observed that, 5 mM 1,10-phenanthroline (in 2% DMSO), a chelator of divalent cations including zinc,[59] inhibited the enzyme activity in the absence of Mg^{2+}, but activated it in the presence of Mg^{2+}.[40] The fact that DMSO inhibits the synthetase activity in the presence of Mg^{2+} is not compatible with a view of interaction between DMSO and the zinc atom(s).

In another study, the IC_{50} value of DMSO for AChE from oyster heart was determined to be 18.2 mM and that for AChE purified from electric eel to be 10 μl/ml or 1% (*ca.* 140 mM).[53] The active site of AChE consists of two distinct subregions; an anionic site to bind the $-N^+(CH_3)_3$ portion and

an esteratic site to split the >C=O (carbonyl) ester bond in the acetylcholine (ACh) molecule. The authors noticed a structural resemblance between DMSO and ACh, and postulated that DMSO competes with the substrate ACh either by binding to the anionic site at its $=S(CH_3)_2$ group or by binding to the esteratic site at the >S=O (sulfinyl) group. The IC_{50} value of DMSO for poly(ADP-ribose) synthetase is about 34% (*ca.* 4760 mM) at 200 μM NAD^+ (Figure 2). This value is much higher than the K_i or IC_{50} values reported for LADH or AChE, indicating that the effect of DMSO on poly(ADP-ribose) synthetase is very weak. In view of the fact that a common structural feature shared by substrate, NAD^+, and many strong inhibitors of poly(ADP-ribose) synthetase is a carbonyl group,[40] it seems possible that the sulfinyl group of DMSO mimics the carbonyl group in the interaction with the enzyme molecule. The same structural feature is shared by two other competitive and weaker inhibitors, 5-nitrouracil and xanthurenic acid.[40]

The present study showed that the inhibition by DMSO is apparently competitive with NAD^+, suggesting more or less interaction of DMSO with the NAD^+ binding site. The very high IC_{50} value of DMSO and the strong inhibition by ethanol and methanol at similar concentrations (> 30%) indicate that DMSO most probably induces a conformational change in the enzyme molecule. This change might modulate the activity directly or indirectly around the catalytic site, and also affect the action of other inhibitors. The effects of DMSO on a variety of enzymes reportedly depend on the structure and function of individual enzyme proteins,[60–72] for many of which conformational changes have been suggested.[73–86]

DMSO and inhibitors of poly(ADP-ribose) synthetase are known to induce differentiation of several tumor cells, including murine erythroleukemia cells,[9,87] teratocarcinoma cells,[10] and HL-60 cells.[44,88] Although the molecular mechanism of their actions is not clearly understood, the inhibition of poly(ADP-ribose) synthetase appears to be, at least, partly involved in the mechanism. In fact, the activity of poly(ADP-ribose) synthetase markedly decreases in cells induced to differentiation,[10,87] and the amount of poly(ADP-ribose) decreases to 15% in DMSO-treated HL-60 cells in 14 h and remains at this low level until the third day.[89] An idea that the action of DMSO on the poly(ADP-ribose) level might be through ADP-ribosyl protein lyase or poly(ADP-ribose) glycohydrolase rather then poly(ADP-ribose) synthetase which is only weakly inhibited by DMSO[44] needs be tested by taking the possibility into account that the effect of DMSO could be stronger in the cell under certain conditions, particularly at lowered NAD^+ concentrations (Figure 1). At present, no data is available for

NAD^+ levels in cells before and after differentiation. The observation that the degree of cell differentiation induced by 1.5% DMSO together with another inhibitor of the synthetase, nicotinamide, at 5–20 mM, is greater than the sum of individual effects[87] may or may not support our view of a common mechanism for DMSO and the synthetase inhibitors; in our *in vitro* assays, 2% DMSO could modulate the inhibitory effect of nicotinamide either negatively or positively at different concentrations (data not shown, cf. Figure 4).

It is our current view that any *in vivo* effect of DMSO, in combination with or without other inhibitor of poly(ADP-ribose) synthetase, should not be simply assigned to the action on a specific enzyme such as the synthetase, but could be most properly assigned to combined actions on multiple enzymes by referring to actual concentrations of DMSO, inhibitor, if any, and substrate(s) such as NAD^+ in the cell.

Acknowledgments

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References

[1] Hayaishi, O. and Ueda, K. (1977) *Annu. Rev. Biochem.*, **46**, 95–116.
[2] Ueda, K., Kawaichi, M. and Hayaishi, O. (1982). In *ADP-Ribosylation Reactions: Biology and Medicine* (Hayaishi, O. and Ueda, K., eds), pp. 117–155. New York: Academic Press.
[3] Mandel, P., Okazaki, H. and Niedergang, C. (1982) *Prog. Nucleic Acid Res. Mol. Biol.*, **27**, 1–51.
[4] Ueda, K. and Hayaishi, O. (1985) *Annu. Rev. Biochem.*, **54**, 73–100.
[5] Althaus, F.R. (1987). In *ADP-Ribosylation of Proteins* (Althaus, F.R. and Richter, C., eds), pp. 1–125. Berlin: Springer-Verlag.
[6] Durkacz, B.W., Omidiji, O., Gray, D.A. and Shall, S. (1980) *Nature*, **283**, 593–596.
[7] Shall, S. (1984) *Adv. Radiat. Biol.*, **11**, 1–69.
[8] Caplan, A.I. and Rosenberg, M.J. (1975) *Proc. Natl. Acad. Sci. USA*, **72**, 1852–1857.
[9] Terada, M., Fujiki, H., Marks, P.A. and Sugimura, T. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 6411–6414.
[10] Ohashi, Y., Ueda, K., Hayaishi, O., Ikai, K. and Niwa, O. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 7132–7136.
[11] Ueda, K., Banasik, M., Nakajima, S., Yook, H.Y. and Kido, T. (1995) *Biochimie*, **77**, 368–373.
[12] Kidwell, W.R. and Burdette, K.E. (1974) *Biochem. Biophys. Res. Commun.*, **61**, 766–773.
[13] Berger, N.A., Kaichi, A.S., Steward, P.G., Klevecz, R.R., Forrest, G.L. and Gross, S.D. (1978) *Exp. Cell Res.*, **117**, 127–135.
[14] Juarez-Salinas, H., Sims, J.L. and Jacobson, M.K. (1979) *Nature*, **282**, 740–741.
[15] Kun, E., Kirsten, E., Milo, G.E., Kurian, P. and Kumari, H.L. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 7219–7223.

[16] Müller, W.E.G., Totsuka, A., Nusser, I., Obermeier, J., Rhode, H.J. and Zahn, R.K. (1974) *Nucleic Acids Res.*, **1**, 1317–1327.
[17] Taniguchi, T., Agemori, M., Kameshita, I., Nishikimi, M. and Shizuta, Y. (1982) *J. Biol. Chem.*, **257**, 4027–4030.
[18] Stone, P.R., Lorimer, W.S. III and Kidwell, W.R. (1977) *Eur. J. Biochem.*, **81**, 9–18.
[19] Wong, M., Malik, N. and Smulson, M. (1982) *Eur. J. Biochem.*, **128**, 209–213.
[20] Poirier, G.G., de Murcia, G., Jongstra-Bilen, J., Niedergang, C. and Mandel, P. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 3423–3427.
[21] Zhang, J., Dawson, V.L., Dawson, T.M. and Snyder, S.H. (1994) *Science*, **263**, 687–689.
[22] Cosi, C., Suzuki, H., Milani, D., Facci, L., Menegazzi, M., Vantini, G., Kanai, Y. and Skaper, S.D. (1994) *J. Neurosci. Res.*, **39**, 38–46.
[23] Sheng, P., Cerruti, C., Ali, S. and Cadet, J.L. (1996) *Ann. N.Y. Acad. Sci.*, **801**, 174–186.
[24] Lam, T.T. (1997) *Res. Commun. Mol. Pathol. Pharmacol.*, **95**, 241–252.
[25] Eliasson, M.J.L., Sampei, K., Mandir, A.S., Hurn, P.D., Traystman, R.J., Bao, J., Pieper, A., Wang, Z.-Q., Dawson, T.M., Snyder, S.H. and Dawson, V.L. (1997) *Nature Med.*, **3**, 1089–1095.
[26] Takahashi, K., Greenberg, J.H., Jackson, P., Maclin, K. and Zhang, J. (1997) *J. Cereb. Blood Flow Metab.*, **17**, 1137–1142.
[27] Endres, M., Wang, Z.-Q., Namura, S., Waeber, C. and Moskowitz, M.A. (1997) *J. Cereb. Blood Flow Metab.*, **17**, 1143–1151.
[28] Cookson, M.R., Ince, P.G. and Shaw, P.J. (1998) *J. Neurochem.*, **70**, 501–508.
[29] Dierenfeldt, S.M., Warnick, C.T. and Lazarus, H.M. (1987) *Biochem. Med. Metab. Biol.*, **38**, 88–103.
[30] Rice, W.G., Hillyer, C.D., Harten, B., Schaeffer, C.A., Dorminy, M., Lackey, D.A. III, Kirsten, E., Mendeleyev, J., Buki, K.G., Hakam, A. and Kun, E. (1992) *Proc. Natl. Acad. Sci. USA*, **89**, 7703–7707.
[31] Kaufmann, S.H., Desnoyers, S., Ottaviano, Y., Davidson, N.E. and Poirier, G.G. (1993) *Cancer Res.*, **53**, 3976–3985.
[32] Thiemermann, C., Bowes, J., Myint, F.P. and Vane, J.R. (1997) *Proc. Nati. Acad. Sci. USA*, **94**, 679–683.
[33] Zingarelli, B., Cuzzocrea, S., Zsengellér, Z., Salzman, A.L. and Szabó, C. (1997) *Cardiovasc. Res.*, **36**, 205–215.
[34] Duriez, P.J. and Shah, G.M. (1997) *Biochem. Cell. Biol.*, **75**, 337–349.
[35] Wang, Z.-Q., Auer, B., Stingl, L., Berghammer, H., Haidacher, D., Schweiger, M. and Wagner, E.F. (1995) *Genes Dev.*, **9**, 509–520.
[36] Wang, Z.-Q., Stingl, L., Morrison, C., Jantsch, M., Los, M., Schulze-Osthoff, K. and Wagner, E.F. (1997) *Genes Dev.*, **11**, 2347–2358.
[37] Cleaver, J.E., Milam, K.M. and Morgan, W.F. (1985) *Radiat. Res.*, **101**, 16–28.
[38] Banasik, M., Komura, H., Saito, I., Abed, N.A.N. and Ueda, K. (1989). In *ADP-Ribose Transfer Reactions: Mechanisms and Biological Significance* (Jacobson, M.K. and Jacobson, E.L., eds), pp. 130–133. New York: Springer-Verlag.
[39] Banasik, M., Komura, H. and Ueda, K. (1990) *FEBS Lett.*, **263**, 222–224.
[40] Banasik, M., Komura, H., Shimoyama, M. and Ueda, K. (1992) *J. Biol. Chem.*, **267**, 1569–1575.
[41] Banasik, M., Komura, H. and Ueda, K. (1992). In *ADP-Ribosylation Reactions* (Poirier, G.G. and Moreau, P., eds), pp. 343–350. New York: Springer-Verlag.
[42] Banasik, M., Shimoyama, M., Moss, J. and Ueda, K. (1992). In *Oji International Seminar on ADP-Ribosylation Reaction: Mechanism and Biological Function*, Abstracts, p. 18. Tsukuba, Japan.
[43] Banasik, M. and Ueda, K. (1994) *Mol. Cell Biochem.*, **138**, 185–197.
[44] Shima, H., Nakayasu, M., Aonuma, S., Sugimura, T. and Nagao, M. (1989) *Proc. Natl. Acad. Sci. USA*, **86**, 7442–7445.
[45] Moss, J., Stanley, S.J. and Watkins, P.A. (1980) *J. Biol. Chem.*, **255**, 5838–5840.
[46] Berger, N.A., Weber, O. and Kaichi, A.S. (1978) *Biochim. Biophys. Acta*, **519**, 87–104.
[47] Bauer, P.I., Hakam, A. and Kun, E. (1986) *FEBS Lett.*, **195**, 331–338.
[48] de la Torre, J.C. (ed.) (1983) *Ann. N. Y. Acad. Sci.*, **411**, 1–404.
[49] Brayton, C.F. (1986) *Cornell Vet.*, **76**, 61–90.

[50] Jacob, S.W. and Herschler, R. (1986) *Cryobiology*, **23**, 14–27.
[51] Yu, Z.-W. and Quinn, P.J. (1994) *Biosci. Rep.*, **14**, 259–281.
[52] Perlman, R.L. and Wolff, J. (1968) *Science*, **160**, 317–319.
[53] Plummer, J.M., Greenberg, M.J., Lehman, H.K. and Watts, J.A. (1983) *Biochem. Pharmacol.*, **32**, 151–158.
[54] Jagota, S.K. (1992) *Indian J. Med. Res.*, **96**, 275–278.
[55] Morgan, E.T., Koop, D.R. and Coon, M.J. (1982) *J. Biol. Chem.*, **257**, 13951–13957.
[56] Cotton, F.A. and Francis, R. (1960) *J. Am. Chem. Soc.*, **82**, 2986–2991.
[57] Zahradka, P. and Ebisuzaki, K. (1984) *Eur. J. Biochem.*, **142**, 503–509.
[58] Gradwohl, G., Ménissier de Murcia, J., Molinete, M., Simonin, F., Koken, M., Hoeijmakers, J.H.J. and de Murcia, G. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 2990–2994.
[59] Vallee, B.L., Williams, R.J.P. and Hoch, F.L. (1959) *J. Biol. Chem.*, **234**, 2621–2626.
[60] Gerhards, E. and Gibian, H. (1967) *Ann. N. Y. Acad. Sci.*, **141**, 65–76.
[61] Monder, C. (1967) *Ann. N. Y. Acad. Sci.*, **141**, 300–301.
[62] Burges, R.A., Blackburn, K.J. and Spilker, B.A. (1969) *Life Sci.*, *8* (p. I), 1325–1335.
[63] Steinschneider, A. and Druck, K. (1972) *Biochim. Biophys. Acta*, **287**, 77–89.
[64] The, R. and Hasselbach, W. (1977) *Eur. J. Biochem.*, **74**, 611–621.
[65] Hoffman, J.L. and Kunz, G.L. (1977) *Biochem. Biophys. Res. Commun.*, **77**, 1231–1236.
[66] Adams, P.A., Baldwin, D.A., Collier, G.S. and Pratt, J.M. (1979) *Biochem. J.*, **179**, 273–280.
[67] Sakamoto, J. (1984) *J. Biochem.* (Tokyo), **96**, 483–487.
[68] Xia, X.-J., Gu, X.-B., Santorelli, A.C. and Yu, R.K. (1989) *J. Lipid Res.*, **30**, 181–188.
[69] Chakravarthy, B.R., Tremblay, R., Macdonald, P., Krsmanovic, V., Whitfield, J.F. and Durkin, J.P. (1992) *Biochim. Biophys. Acta*, **1136**, 83–90.
[70] Huang, J., Nasr, M., Kim, Y. and Matthews, H.R. (1992) *J. Biol. Chem.*, **267**, 15511–15515.
[71] Morbach, S., Tebbe, S. and Schneider, E. (1993) *J. Biol. Chem.*, **268**, 18617–18621.
[72] Harwood, H.J. Jr. (1995) *Anal. Biochem.*, **226**, 268–278.
[73] Hamaguchi, K. (1964) *J. Biochem.* (Tokyo), **56**, 441–449.
[74] Rammler, D.H. (1967) *Ann. N. Y. Acad. Sci.*, **141**, 291–299.
[75] Chang, C.-Y. and Simon, E. (1968) *Proc. Soc. Exp. Biol. Med.*, **128**, 60–66.
[76] Masson, A. and Wüthrich, K. (1973) *FEBS Lett.*, **31**, 114–118.
[77] Cooney, C.L. and Hueter, J. (1974) *Biotechnol. Bioeng.*, **16**, 1045–1053.
[78] Jacobson, A.L. and Krueger, P.J. (1975) *Biochim. Biophys. Acta*, **393**, 274–283.
[79] Tritsch, G.L., Niswander, P.R., Rosenfeld, J., Nechaev, A. and Mittelman, A. (1976) *Mol. Cell. Biochem.*, **12**, 93–101.
[80] Jacobson, A.L. and Turner, C.L. (1980) *Biochemistry*, **19**, 4534–4538.
[81] Durán, N., Baeza, J., Freer, J., Brunet, J.E., Gonzalez, G.A., Sotomayor, C.P. and Faljoni-Alario, A. (1981) *Biochem. Biophys. Res. Commun.*, **103**, 131–138.
[82] Swann, A.C. (1983) *J. Biol. Chem.*, **258**, 11780–11786.
[83] Buchet, R., Jona, I. and Martonosi, A. (1989) *Biochim. Biophys. Acta*, **983**, 167–178.
[84] Jackson, M. and Mantsch, H.H. (1991) *Biochim. Biophys. Acta*, **1078**, 231–235.
[85] Ryan, J.J. and Clarke, M.F. (1994) *Leukemia Res.*, **18**, 617–621.
[86] Bhattacharjya, S. and Balaram, P. (1997) *Proteins*, **29**, 492–507.
[87] Morioka, K., Tanaka, K., Nokuo, T., Ishizawa, M. and Ono, T. (1979) *Gann*, **70**, 37–46.
[88] Grosso, L.E. and Pitot, H.C. (1984) *Biochem. Biophys. Res. Commun.*, **119**, 473–480.
[89] Kanai, M., Miwa, M., Kondo, T., Tanaka, Y., Nakayasu, M. and Sugimura, T. (1982) *Biochem. Biophys. Res. Commun.*, **105**, 404–411.