Inhibition of poly(ADP-ribose) synthetase by unsaturated fatty acids, vitamins and vitamin-like substances

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Various vitamins and vitamin-like substances inhibited the activity of poly(ADP-ribose) synthetase in vitro. The most potent were essential fatty acids, i.e. arachidonic acid, linoleic acid, and linolenic acid; their 50% inhibitory concentrations (IC_{50}) were 44–110 μM, indicating a higher potency than nicotinamide, a well-known vitamin inhibitor ($IC_{50} = 210$ μM). Vitamins K_3, K_1, and retinal were the next strongest inhibitors, followed by α-lipoic acid, coenzyme Q_0, and pyridoxal 5-phosphate. Nicotinamide and vitamin K_3 exhibited mixed-type inhibition with respect to NAD^+, while arachidonic acid exhibited dual inhibitions, competitive at 50 μM and mixed-type at 100 μM.

Poly(ADP-ribose) synthesis; Enzyme inhibition; Vitamin; NAD; Arachidonate; ADP-ribosyltransferase

1. INTRODUCTION

Poly(ADP-ribose) synthetase (EC 2.4.2.30) is a nuclear enzyme that catalyzes DNA-dependent transfers of the ADP-ribose moiety of NAD^+ to an acceptor protein (chain initiation) and then to this protein-bound ADP-ribose (chain elongation) with a concomitant release of nicotinamide [1]. Various nuclear proteins, including histones, high-mobility group nonhistone proteins, and the enzyme itself [2,3], have been identified as acceptors of ADP-ribose in vitro as well as in vivo [1]. Many lines of evidence have suggested implications of poly(ADP-ribose) in DNA repair [4], cell differentiation [5,6], transformation [7], transcription [8], and formation of chromatin architecture [9,10]. These biological functions are most probably regulated by some cellular factors, which remain to be found; so far, only DNA strand breaks have been known as a stimulator of the activity [4]. In search of other regulators, we took notice of the inhibitory effects of nicotinamide, benzamide (a constituent of folic acid), and 1,4-naphthoquinone (a parent compound of vitamin Ks) [11], and examined various vitamins and vitamin-like substances. We report here notable effects of unsaturated fatty acids, several vitamins and their derivatives on the synthetase activity.

2. EXPERIMENTAL

2.1. *Enzymes and chemicals*

Poly(ADP-ribose) synthetase was purified from calf thymus as previously described [12]. The enzyme was also purified from bovine thymus by Dr Y. Ohashi (Nara Medical College), and kindly donated to us. [*Ade*-^{14}C]NAD^+ was obtained from Amersham International. All compounds tested for inhibition were of the highest grade commercially available. The purity (~100%) of vitamin D_2 and α-lipoic acid was confirmed by thin-layer chromatography and proton nuclear magnetic resonance; the purity (>85%) of retinal was estimated by UV/VIS absorption and high-performance liquid chromatography.

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2.2. *Poly(ADP-ribose) synthetase assay*

The synthetase activity was assayed by measuring incorporation of ^{14}C from [*Ade*-^{14}C]NAD^+ into Cl_3CCOOH-insoluble material. The reaction mixture (200 μl) containing 100 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 5 mM dithiothreitol, 32.5 μg/ml calf thymus DNA (sheared by sonication ten times for 10 s), 200 μM [^{14}C]NAD^+ ($1.3–2.6 \times 10^5$ cpm), enzyme (1.8 μg), and, if any, test compound was incubated for 10 min at 37°C. The reaction was terminated by the addition of 20% Cl_3CCOOH. Acid-insoluble material was collected on a nitrocellulose filter (Millipore; HA type) or a glas fiber filter (Whatman; GF/C), and the filter was washed with 5% Cl_3CCOOH and examined for ^{14}C. Mode of inhibition was examined under standard conditions except the use of bovine thymus enzyme (1.5 μg) and the incubation for 30 s at 25°C. Compounds that were not completely water-soluble at ≥10 mM were first dissolved in dimethylsulfoxide (Me_2SO), and added into the reaction mixture. Quenching of scintillation by colored compounds was corrected for by the internal standardization method.

3. RESULTS AND DISCUSSION

Various vitamins and derivatives proved to be inhibitory to the poly(ADP-ribose) synthetase activity (Table IA). Nicotinamide, a well-known inhibitor of this enzyme, was the strongest among vitamins established for humans. This compound, though documented as a competitive inhibitor [13], exhibited mixed-type inhibition with respect to NAD^+ in our system (Fig. 1A). We confirmed that a synthetic

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Table I

Effects of various vitamins and vitamin-like compounds on poly(ADP-ribose) synthetase activity

Compound	IC_{50} (μM)	Inhibition (%) at	
		1 mM	5 mM
(A)			
Vitamin A (retinol)[a]	–	15	–
Vitamin A acid (retinoic acid)[a]	–	>30[c]	–
Vitamin A aldehyde (retinal)[a]	450	>70	–
Vitamin B_1 hydrochloride	–	−1[d]	8
Vitamin B_2[a]	–	13	–
Vitamin B_6 hydrochloride	–	4	10
Pyridoxal 5-phosphate[a]	4250	13	56
D-Biotin[a]	–	3	8
Folic acid[a]	–	12	–
Nicotinic acid	–	3	9
Nicotinamide	210	72	87
D-Pantothenic acid sodium salt	–	2	1
Vitamin B_{12}[a]	–	>15	–
Vitamin C	–	−1	8
Vitamin D_2 (calciferol)[a]	–	>9	>13
Vitamin D_3 (cholecalciferol)[a]	–	>3	>7
Vitamin E (DL-α-tocopherol)[a]	–	>21	>43
Vitamin K_1 (phytomenadione)[b]	520	>72	–
Vitamin $K_{2(20)}$ (menatetrenone)[a]	–	−5	>25
Vitamin K_3 (menadione)[a]	420	>70	>84
(B)			
p-Aminobenzoic acid sodium salt	–	2	7
Arachidonic acid[a]	44	>67	–
DL-Carnitine chloride	–	0	5
Choline chloride	–	5	8
Coenzyme Q_0	3900	−2	79
Hesperidin (vitamin P)[a]	–	1	26
Inositol	–	−3	0
Linoleic acid[a]	48	85	–
Linolenic acid[a]	110	>70	–
DL-α-Lipoamide[a]	–	2	>9
DL-α-Lipoic acid (oxidized form)[a]	–	3	34
DL-α-Lipoic acid (reduced form)[a]	–	58	–
DL-Methionine-S-methylsulfonium (vitamin U) chloride	–	8	11
Orotic acid	–	6	24

[a] 2% (final) Me_2SO
[b] 10% (final) Me_2SO
[c] Minimum value estimated under conditions of limited solubility
[d] Stimulation

vitamin, K_3, was a fairly strong inhibitor [14], and found that retinal (predominantly all-*trans*) and vitamin K_1 were also fairly strong inhibitors. The potency of vitamin K_1 and other water-insoluble vitamins might be underestimated in the presence of Me_2SO; this solvent, a weak inhibitor by itself, generally reduces effects of other inhibitors (M. Banasik and K. Ueda, unpublished data). Pyridoxal 5-phosphate and vitamin E were weaker inhibitors. Kinetic analysis indicated that vitamin K_3 exhibited mixed-type inhibition with respect to NAD^+ (data not shown).

Some vitamin-like compounds were also inhibitory to poly(ADP-ribose) synthetase (Table IB). Arachidonic acid was the most potent among compounds tested in this study. It is noteworthy that this compound acted as a competitive inhibitor at 50 μM, while as a mixed-type inhibitor at 100 μM (Fig. 1B). Spectrophotometric analysis indicated that arachidonic acid worked in a micelle form, rather than a monomeric form, in the reaction mixture, and the micelle state seemed to differ at 50 and 100 μM (data not shown). A sharp increase in inhibition between 25 and 50 μM (Fig. 2) was in accord with the micelle formation in this concentration range. Two other essential fatty acids, i.e. linoleic acid and linolenic acid, were also strong inhibitors. Their IC_{50} values indicated that they were more potent than nicotinamide. Essentially no inhibition was observed with stearic acid, a saturated fatty acid, up to 5 mM (data not shown). The reduced form of α-lipoic acid exhibited 58% inhibition at 1 mM, whereas the oxidized form exhibited a much weaker effect even at 5 mM under standard conditions. When assayed without Mg^{2+}, however, the oxidized form inhibited 81% of the activity at 5 mM, whereas the reduced form inhibited only 18% at 1 mM. This marked difference in the presence and absence of Mg^{2+} indicated that the reduced form of α-lipoic acid work-

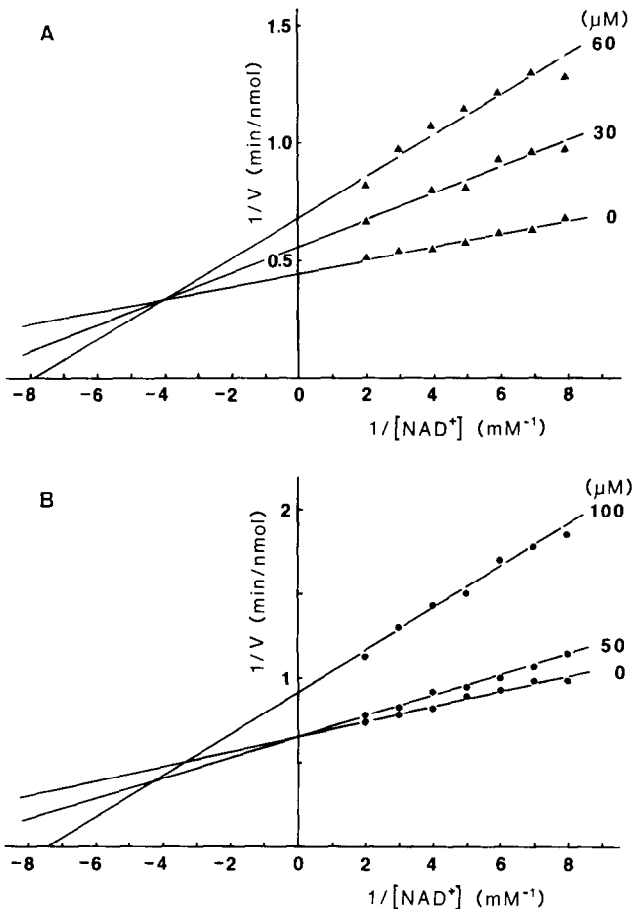

Fig. 1. Modes of inhibition of poly(ADP-ribose) synthetase activity by nicotinamide (A) and arachidonic acid (B).

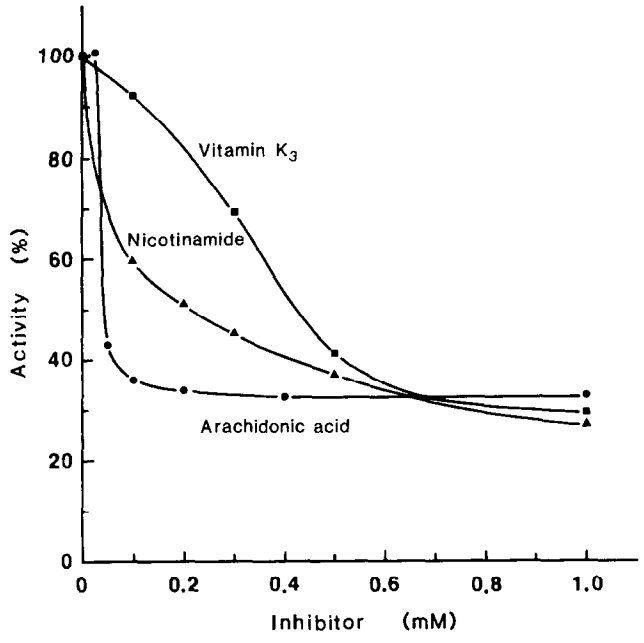

Fig. 2. Inhibition of poly(ADP-ribose) synthetase activity by vitamin K_3, nicotinamide, and arachidonic acid. Activity of 100% (no inhibition) represents incorporation of 1.4 nmol ADP-ribose under standard conditions.

ed, at least partly, through chelation of this activator ion. Coenzyme Q_0, a ubiquinone, was another inhibitor.

The mechanisms of physiological (or slow) actions of water-soluble vitamins are well understood, but those of lipophilic vitamins are not. The mechanisms of pharmacological (or rapid) actions of many vitamins also remain to be elucidated. Recent findings of direct effects of vitamins on certain enzymes may give a clue to these unknown mechanisms; for example, retinal, retinoic acid, and unsaturated fatty acids, at a concentration range of 10–100 μM, activate [15–17], while vitamin E inhibits the protein kinase C activity in vitro [18]. In the present study, we found another direct action of vitamins, i.e. inhibition of the poly(ADP-ribose) synthetase activity.

Whether our in vitro observations have any physiological significance awaits further investigation of metabolism, transport, and, in particular, local concentrations of vitamins. Another useful approach to this problem might be analysis of cultured cells. Our preliminary experiments have shown that retinal, nicotinamide, arachidonic acid, and α-lipoic acid induce murine teratocarcinoma cell differentiation into epitheloid cells in culture, though less efficiently than retinoic acid [6] (K. Ueda and M. Banasik, unpublished results). Thus it seems possible that these vitamins share, at least partly, a common mechanism, such as suppression of poly(ADP-ribose) synthesis, in induction of cell differentiation.

In addition to poly(ADP-ribose) synthetase examined in this study, an arginine- and monomer-specific ADP-ribosyltransferase from hen liver [19] has been found to be inhibited by several vitamins (e.g. K_3) or vitamin-like substances (e.g. arachidonic acid), but not by others (e.g. nicotinamide) (M. Banasik, H. Komura, M. Shimoyama, and K. Ueda, manuscript in preparation). These results may shed light on intricate actions of vitamins or related molecules to the cell through modulation of different types of ADP-ribosylation reactions.

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