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The Cyanogenic Glucoside, Prunasin (D-Mandelonitrile-β-D-Glucoside), Is a Novel Inhibitor of DNA Polymerase β[1]

Yoshiyuki Mizushina,* Naoko Takahashi,* Akitsu Ogawa,* Kyoko Tsurugaya,* Hiroyuki Koshino,† Masaharu Takemura,‡ Shonen Yoshida,‡ Akio Matsukage,§ Fumio Sugawara,* and Kengo Sakaguchi,*,[2]

*Department of Applied Biological Science, Science University of Tokyo, 2641 Yamazaki, Noda, Chiba 278-8510; †The Institute of Physical and Chemical Research (RIKEN), Wako, Saitama 351-0198; ‡Laboratory of Cancer Cell Biology, Research Institute for Disease Mechanism and Control, Nagoya University School of Medicine, Nagoya, Aichi 466-8560; and §Laboratory of Cell Biology, Aichi Cancer Center Research Institute, Nagoya, Aichi 464-8681

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A DNA polymerase β (pol. β) inhibitor has been isolated independently from two organisms; a red perilla, *Perilla frutescens*, and a mugwort, *Artemisia vulgaris*. These molecules were determined by spectroscopic analyses to be the cyanogenic glucoside, D-mandelonitrile-β-D-glucoside, prunasin. The compound inhibited the activity of rat pol. β at 150 μM, but did not influence the activities of calf DNA polymerase α and plant DNA polymerases, human immunodefficiency virus type 1 reverse transcriptase, calf terminal deoxynucleotidyl transferase, or any prokaryotic DNA polymerases, or DNA and RNA metabolic enzymes examined. The compound dose-dependently inhibited pol. β activity, the IC_{50} value being 98 μM with poly dA/oligo dT_{12-18} and dTTP as the DNA template and substrate, respectively. Inhibition of pol. β by the compound was competitive with the substrate, dTTP. The inhibition was enhanced in the presence of fatty acid, and the IC_{50} value decreased to approximately 40 μM. In the presenceof C_{10}-decanoic acid, the K_i value for substrate dTTP decreased by 28-fold, suggesting that the fatty acid allowed easier access of the compound to the substrate-binding site.

Key words: cyanogenic glucoside, enzyme inhibitor, fatty acid, DNA polymerase β, prunasin.

Eukaryotic cells contain multiple species of DNA polymerases, *i.e.* DNA polymerase α, β, γ, δ, ε, ζ, and η (*1-3*). Current intense interest in understanding of the precise *in vivo* roles of these polymerases and of the factors controlling their activity prompted us to undertake a major search for wide inhibitors of these enzymes (*4-13*). We established an assay method (*14, 15*) for detecting DNA polymerase inhibitors and have used it to screen natural products derived from mushrooms, algae and higher plants. We have found and reported new species of inhibitors, such as cerebrosides (*16*), terpenoids (*17-19*), sulfate-containing glycolipids (*20-22*) and fatty acids (*15, 23*). In these previous studies, we used calf thymus DNA polymerase α (pol. α) as a representative replicative DNA polymerase and rat DNA polymerase β (pol. β) as a representative repair-related DNA polymerase, because the other DNA polymerase species were not used routinely at that time. All of the inhibitors found hardly influenced the activities of prokaryotic DNA or RNA metabolic enzymes such as *Escherichia coli* DNA polymerase I, T4 DNA polymerase, Taq DNA polymerase, T7 RNA polymerase, T4 polynucleotide kinase, and deoxyribonuclease I (*15-16, 18-22*). Since the activities of only eukaryotic DNA polymerases such as pol. α, pol. β, terminal deoxynucleotidyl transferase and human immunodefficiency virus type 1 reverse transcriptase were affected, these molecules should be called selective inhibitors of eukaryotic DNA polymerases. They did not show specific inhibitory effects on particular species of DNA polymerases.

We believe at present that a search for pol. β inhibitors is especially urgent, because the role of pol. β *in vivo* remains obscure. Although pol. β has been implicated in short-patch DNA synthesis in the repair process after base excision (*24*), it must have other roles, for example, in DNA repair in meiosis (*9*) and V(D)J recombination (*25*). DideoxyTTP (ddTTP) is well-known as a pol. β inhibitor (*26*). However, ddTTP cannot be used for *in vivo* experiments, because it cannot penetrate into cells. Other compounds reported to inhibit the pol. β activity include fatty acids (*15, 23*), terpenoids (*17-19*), nucleotide analogs (*25-27*), flavonoids (*28, 29*), sulfate- or sialic acid-containing glycolipids (*20-22, 30*), and phospholipids (*31, 32*), but these agents inhibited the activities of both pol. α and pol. β.

Thus, we screened for a pol. β-specific inhibitor, and found a natural compound that inhibits mammalian pol. β activity but not pol. α activity in the two sources; the red perilla, *Perilla frutescens*, and the mugwort, *Artemisia vulgaris*. The compound was determined to be D-mandelonitrile-β-D-glucoside (prunasin), a cyanogenic glucoside,

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[2] To whom correspondence should be addressed. Phone: +81-471-24-1501, Fax: +81-471-23-9767, E-mail: kengo@rs.noda.sut.ac.jp

previously reported to be a plant growth inhibitor (*33, 34*). We report here the isolation, spectroscopic analyses and biochemical action of prunasin, in comparison with its analog, D-mandelonitrile-β-D-gentiobioside (amygdalin) (*35*).

MATERIALS AND METHODS

Materials—Nucleotides and chemically synthesized template-primers such as poly(dA) and oligo(dT)$_{12-18}$ were purchased from Pharmacia (Uppsala, Sweden). [^{3}H]Deoxythymidine 5′-triphosphate (dTTP) (43 Ci/mmol) was purchased from New England Nuclear (Boston, MA, USA). Amygdalin was purchased from Sigma (St. Louis, MO, USA). The fatty acids were named using the nomenclature described by Weete (*36*). In the following, (A:B), "A" refers to the number of carbon atoms and "B" to the number of double bonds. For example, the straight fatty acid containing 10 carbon atoms and no double bonds, capric acid, was designated as 10:0. The following fatty acids were purchased from Nu Check Prep: *n*-hexanoic acid (capronic acid, 6:0), *n*-octanoic acid (caprylic acid, 8:0), *n*-decanoic acid (capric acid, 10:0), *n*-dodecanoic acid (lauric acid, 12:0), *n*-tetradecanoic acid (myristic acid, 14:0), *n*-hexadecanoic acid (palmitic acid, 16:0), *n*-octadecanoic acid (stearic acid, 18:0), *cis*-11-dodecenoic acid (12:1), *cis*-9-tetradecenoic acid (myristoleic acid, 14:1), *cis*-9-hexadecenoic acid (palmitoleic acid, 16:1), and *cis*-9-octadecenoic acid (oleic acid, 18:1). All other reagents were of analytical grade and were purchased from Wako Chemical Industries (Osaka). The red perilla, *Perilla frutescens*, and the mugwort, *Artemisia vulgaris*, were collected from fields in the vicinity of Noda City, Chiba Prefecture.

Enzymes and DNA Polymerase Assays—The DNA polymerases and DNA metabolic enzymes used, and the enzyme assay methods were the same as those described previously (*14, 15, 37-41*). One unit of each DNA polymerase activity was defined as the amount of enzyme that catalyzed the incorporation of 1 nmol of deoxyribonucleoside triphosphates (*i.e.*, dTTP) into synthetic template-primers [*i.e.*, poly(dA)/oligo(dT)$_{12-18}$, A/T = 2:1] in 60 min at 37°C under the normal reaction conditions for each enzymes (*14, 15*). The activities of T7 RNA polymerase and T4 polynucleotide kinase were measured by means of standard assays according to the manufacturer's specifications, as described Nakayama and Saneyoshi (*37*) and Soltis and Uhlenbeck (*38*), respectively. The activity of bovine deoxyribonuclease I was measured by the methods described in previous reports (*39-41*). Prunasin was dissolved in dimethylsulfoxide (DMSO), and 4 μl of the dissolved sample was mixed with 16 μl of each enzyme (final, 0.05 unit) in 50 mM Tris-HCl (pH 7.5) containing 0.1 mM EDTA, 1 mM dithiothreitol, and 50% glycerol, and then kept at 0°C for 10 min. Eight microliters of each of the preincubated solutions was added to 16 μl of each of the enzyme standard reaction mixtures, and then the enzyme activities were measured.

Extraction and Purification of Prunasin from the Red Perilla, Perilla frutescens—Leaves of the red perilla, *Perilla frutescens* (300 g), were homogenized with a Waring blender, and then extracted with acetone for 3 days. The evaporated extract was partitioned between EtOAc and H_2O and then adjusted to pH 7. The evaporated organic layer was subjected to silica gel column chromatography and then eluted with $CHCl_3$:MeOH:H_2O (v/v/v 10:1:0.1). The active fractions were collected and then purified by second silica gel column chromatography using EtOAc:MeOH:H_2O (v/v/v 20:1:0.1), the active fractions being collected. The collected fractions were purified by third silica gel column chromatography using EtOAc:MeOH:H_2O (v/v/v 10:1:0.1). The active fractions (prunasin) were finally purified by gel filtration column chromatography on Sephadex LH-20 to give a white powder (8 mg).

Extraction and Purification of Prunasin from the Mugwort, Artemisia vulgaris—The mugwort, *A. vulgaris*, was homogenized and extracted with acetone (300 g). The evaporated extract was partitioned between EtOAc and H_2O, and then adjusted to pH 7. The evaporated organic layer was subjected to silica gel column chromatography and then eluted with EtOAc:MeOH:H_2O (v/v/v 50:1:0.1). The active fractions were collected and then purified by second silica gel column chromatography using $CHCl_3$:MeOH (v/v 8:1) the active fractions being collected (30 mg). The active fractions (prunasin) were finally purified by gel filtration column chromatography on Sephadex LH-20 to give a white powder (25 mg).

RESULTS AND DISCUSSION

The search for eukaryotic DNA polymerase inhibitors is important, because at present organisms defective in each of the enzymes are not available. We have been strongly interested in DNA polymerase β (pol. β)-specific inhibitors, because many of the roles of pol. β *in vivo* remain obscure. DideoxyTTP (ddTTP), the only well-known pol. β inhibitor, cannot be used for *in vivo* experiments, because it cannot penetrate into cells. We screened for a pol. β-specific inhibitor, and found a compound in the extracts of two plants, the red perilla, *P. frutescens*, and the mugwort, *A. vulgaris*, with such inhibitory activity.

Identification of Natural Compounds from the Red Perilla, P. frutescens, and the Mugwort, A. vulgaris—As described under "MATERIAL AND METHODS", the purified compounds from the red perilla and mugwort were each obtained as a white powder (8 and 25 mg, respectively). Each compound was analyzed by spectrometric means. The data for the red perilla and mugwort compounds revealed they were chemically the same, the compound being a cyanogenic glucoside, D-mandelonitrile-β-D-glucoside (prunasin), reported to be a plant growth inhibitor (*33, 34*). Prunasin is an analog of a material known as amygdalin, a plant cytotoxic agent reported originally in 1923 (*35*) (Fig. 1). Figure 1 shows the chemical structures of prunasin, D-mandelonitrile-β-D-glucoside (A), and its analog, amygdalin, D-mandelonitrile-β-D-gentiobioside (B). We examined the influence of prunasin on DNA polymerases and DNA metabolic enzymes.

Effects of Prunasin on Various DNA Polymerases and DNA Metabolic Enzymes—As shown in Table I, among the enzymes examined (eukaryotic DNA polymerases α and β, prokaryotic DNA polymerases such as the Klenow fragment of DNA polymerase I, T4 DNA polymerase, and Taq DNA polymerase, and DNA metabolic enzyme activities such as T7 RNA polymerase, T4 polynucleotide kinase, bovine deoxyribonuclease I, calf thymus terminal deoxynucleotidyl transferase, or human immunodeficiency virus

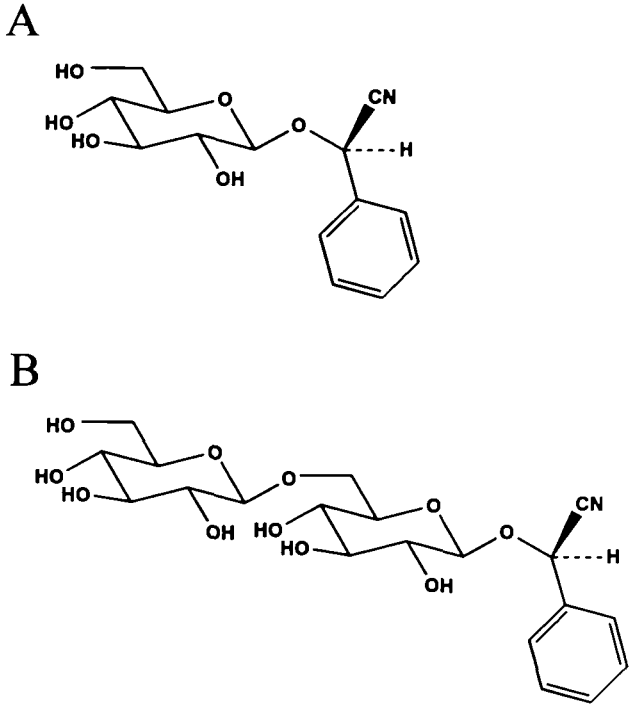

Fig. 1. **Chemical structures of prunasin from the red perilla, *Perilla frutescens*, and the mugwort, *Artemisia vulgaris* (A) and its analog, amygdalin (B).**

type 1 reverse transcriptase), prunasin at 150 μM only inhibited the activity of rat pol. β. The compound seemed to slightly inhibit the activity of calf pol. α (Fig. 2), but this effect was not significant (Table I). Prunasin did not only influence pol. α activity, but also the activities of cauliflower DNA polymerases I (plant alternative of pol. α) and II (plant alternative of pol. β), prokaryotic DNA polymerases, and DNA metabolic enzymes. Each of their IC_{50} values was over 500 μM (Table I). This suggested that prunasin is a pol. β-specific inhibitor, although the effects of this compound on pol. γ, δ, ε, ζ, and η remain to be investigated. Since these DNA polymerases cannot be obtained readily, we will refer to prunasin as a rat pol. β inhibitor, not a pol. β-specific inhibitor, in this report. Despite its structural similarity to prunasin, amygdalin did not influence the activities of any of the enzymes examined (Table I).

The IC_{50} value of prunasin toward rat pol. β activity was 98 μM (Table I and Fig. 2). Since the IC_{50} of ddTTP for this enzyme was 15 to 20 μM under the same conditions (*25*), prunasin seemed not to be such a strong inhibitor. However, the active fractions of the plant extracts containing prunasin at each of the purification steps before the respective second or third silica gel column chromatography originally seemed inhibit pol. β much more strongly than the final purified one. During the process of purification, therefore, some as yet unidentified factors which may enhance the inhibitory effect of prunasin may have been released from the extracts. We reported a similar phenomenon in a previous investigation on long-chain fatty acids as DNA polymerase inhibitors (*42*). We reported previously that fatty acids longer than C_{18}, especially the unsaturated forms, could act as potent inhibitors of pol. α and β, and

TABLE I. **IC_{50} values of prunasin and amygdalin for the activities of various of DNA polymerases and other DNA metabolic enzymes.**

Enzyme	Prunasin (μM)	Amygdalin (μM)
Calf DNA polymerase α	>500	>500
Rat DNA polymerase β	98	>500
Plant DNA polymerase I (α like)	>500	>500
Plant DNA polymerase II (β like)	>500	>500
E. coli DNA polymerase I (Klenow fragment)	>500	>500
Taq DNA polymerase	>500	>500
T4 DNA polymerase	>500	>500
Calf terminal deoxynucleotidyl transferase	>500	>500
HIV-1 reverse tramscroptase	>500	>500
T7 RNA polymerase	>500	>500
T4 polynucleotide kinase	>500	>500
Bovine deoxyribonuclease I	>500	>500

Prunasin and amygdalin were incubated with each enzyme (0.05 unit each). The enzymatic activity was measured as described under "MATERIALS AND METHODS". Enzyme activity (5,000 cpm) without prunasin and amygdalin was taken as 100%.

conversely that fatty acids with chains shorter than C_{14} have no such effect (*15*). An ergosterol peroxide, although the agent itself had no such inhibitory effect, only enhanced the pol. β-inhibition by linoleic acid (*42*). We proposed that this enhancement was due to a conformational change in the pol. β protein structure caused by the fatty acid and the ergosterol peroxide (*42*). Therefore, we analyzed the inhibition by prunasin in the absence or presence of various fatty acids.

Inhibition by Prunasin of the Activities of DNA Polymerases with or without Fatty Acids—Each of the linear chain fatty acids (Table II shows C_6 to C_{18} saturated fatty acids and C_{12} to C_{18} mono-unsaturated fatty acids) at 10 μM was added to 25 or 75 μM prunasin, and then its inhibitory effects were investigated. Fatty acids shorter than C_{14} at all concentrations examined and fatty acids longer than C_{16} at 10 μM did not inhibit the activity of pol. β (*15, 23*). As shown in Table II, 25 or 75 μM prunasin caused 2.2 or 38.4% inhibition of pol. β, respectively. Interestingly, the fatty acids enhanced the inhibitory effect of prunasin, and the degree of enhancement changed with the species of fatty acid (Table II). Capric acid (C_{10}, 10:0), a middle chain fatty acid, was the strongest enhancer of the inhibition of pol. β (Table II). The pol. β activity increased more than 2-fold on the addition of capric acid at 75 μM (Table II). Among the saturated fatty acids examined, the fatty acids longer than C_{16} had no effect (Table II). None of the saturated fatty acids themselves examined were inhibitors of pol. β (*15*), the pol. β-inhibition by these fatty acids alone was found not to occur as shown in Table II. On the other hand, among the C_{12} to C_{18} mono-unsaturated fatty acids, those with longer chains were stronger enhancers of the inhibition of pol. β, and among those tested a long chain fatty acid, oleic acid (C_{18}, 18:1), had the strongest effect (Table II).

We next chose capric acid (C_{10}, 10:0) and oleic acid (C_{18}, 18:1) as the strongest enhancers, and investigated their inhibitory effect more precisely. Figure 2 shows the dose response curves of the effect of prunasin with 10 or 100 μM capric acid or 10 μM oleic acid on pol. β activity. The inhibition by prunasin was dose-dependent, with 50% inhibition of pol. β being observed at the dose of 98 μM.

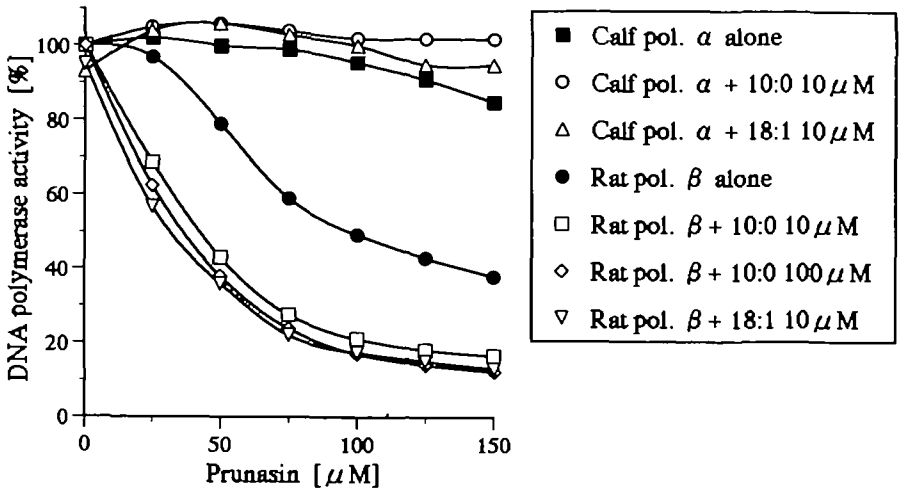

Fig. 2. **Inhibition of pol. α and β activities by prunasin alone or prunasin with capric acid (C_{10}, 10:0) or oleic acid (C_{18}, 18:1).** Calf DNA polymerase α (pol. α) and the indicated concentrations of prunasin were preincubated with 0 (■) or 10 μM capric acid (⊃), or 10 μM oleic acid (△). Rat DNA polymerase β (pol. β) and prunasin were preincubated with 0 (●), 10 μM (□) or 100 μM capric acid (◇) or 10 μM oleic acid (▽). Each mixture contained 0.05 units of enzyme. The enzyme activity (5,000 cpm) without both prunasin and a fatty acid was taken as 100%.

TABLE II. **Inhibition of pol. β activity by mixtures of prunasin and C_6-C_{18} fatty acids.**

	DNA polymerase β activity (%)					
	None		Prunasin (25 μM)		Prunasin (75 μM)	
	Without fatty acid[a]	With fatty acid	Without fatty acid	With fatty acid[b]	Without fatty acid	With fatty acid[c]
	100		97.8		61.6	
Saturated fatty acid (10 μM)						
Capronic acid, 6:0		102		84.3 (1.16)		74.3 (0.83)
Caprylic acid, 8:0		101		83.1 (1.18)		46.6 (1.32)
Capric acid, 10:0		100		80.7 (1.21)		28.9 (2.13)
Lauric acid, 12:0		101		83.0 (1.18)		32.8 (1.88)
Myristic acid, 14:0		100		87.0 (1.12)		65.0 (0.95)
Palmitic acid, 16:0		102		100 (0.98)		100 (0.62)
Stearic acid, 18:0		100		100 (0.98)		100 (0.62)
Unsaturated fatty acid (10 μM)						
cis-11-Dodecenoic acid, 12:1		101		94.0 (1.04)		57.4 (1.07)
Myristoleic acid, 14:1		100		85.2 (1.15)		47.4 (1.30)
Palmitoleic acid, 16:0		100		65.4 (1.50)		35.9 (1.72)
Oleic acid, 18:1		101		56.7 (1.72)		22.3 (2.76)

[a]Enzyme activity (5,000 cpm) without both prunasin and a fatty acid was taken as 100%. [b]The values for [Prunasin (25 μM)]/[Fatty acid (10 μM)+Prunasin (25 μM)]. [c]The values for [Prunasin (75 μM)]/[Fatty acid (10 μM)+Prunasin (75 μM)].

However, the pol. α-inhibitory activity of prunasin was weak. As described previously (*15*), oleic acid itself at 80 μM completely inhibited the activities of pol. α and pol. β, and at less than 25 μM, the fatty acid lost this ability. Capric acid itself does not affect the activity of either pol. α or β (*15*). The fatty acids could increase the pol. β-inhibitory activity of prunasin to approximately 3-fold, as shown in Fig. 2. On the other hand, the presence of either of the fatty acids hardly influenced the effect of prunasin on pol. α, or seemed to antagonize pol. β (Fig. 2). The inhibitory effect of prunasin with either of the fatty acids was dose-dependent, and 10 or 100 μM capric acid or 10 μM oleic acid resulted in 50% inhibition of pol. β at the doses of 45, 37, and 32 μM, respectively (Fig. 2). Prunasin was a potent rat pol. β inhibitor in the presence of either of these fatty acids.

Mode of Pol. β Inhibition by Prunasin with or without Fatty Acid—To elucidate the action of prunasin with fatty acid on pol. β, the extent of inhibition as a function of the DNA template-primer or nucleotide substrate concentration was studied. The influence of capric acid (C_{10}, 10:0) is shown in Fig. 3. For kinetic analysis, poly(dA)/oligo-$(dT)_{12-18}$, A/T=2/1, and nucleotide dTTP were used as the DNA template-primer and substrate, respectively. Double reciprocal plots of the results showed that the inhibition of pol. β by prunasin was noncompetitive with the DNA template (Fig. 3A) and competitive with the substrate (Fig. 3B). The K_m for the DNA template was unchanged at a concentration of 3.3 μM in the presence of prunasin, while its V_{max} decreased from 72 to 20 pmol/h in the presence of 0 to 150 μM prunasin (Fig. 3A). In contrast, the inhibition was competitive with the substrate (Fig. 3B). The apparent K_m for the substrate, dTTP, increased by 3.2-fold in the presence of 0 to 150 μM prunasin, whereas the V_{max} was unchanged at 100 pmol/h (Fig. 3B). The inhibition constant (K_i) values, obtained from Dixon plots, were 45 and 140 μM for the template DNA and substrate dTTP, respectively (Fig. 3, C and D). Prunasin inhibited pol. β throngh a mechanism similar to that in the case of ddTTP (*25, 26*). However, while ddTTP cannot penetrate into viable cells, prunasin does so easily (*43*). Therefore, prunasin could be a substance similar to a cell-permeable ddTTP.

The inhibition of pol. β by the mixture of prunasin and capric acid was also noncompetitive with the DNA template, since there were no changes in the apparent K_m (3.3 μM), while the V_{max} decreased from 70 to 17 pmol/h, in the presence of 0 to 60 μM prunasin with 10 μM capric acid (Fig. 3E). Whereas the apparent V_{max} for the substrate

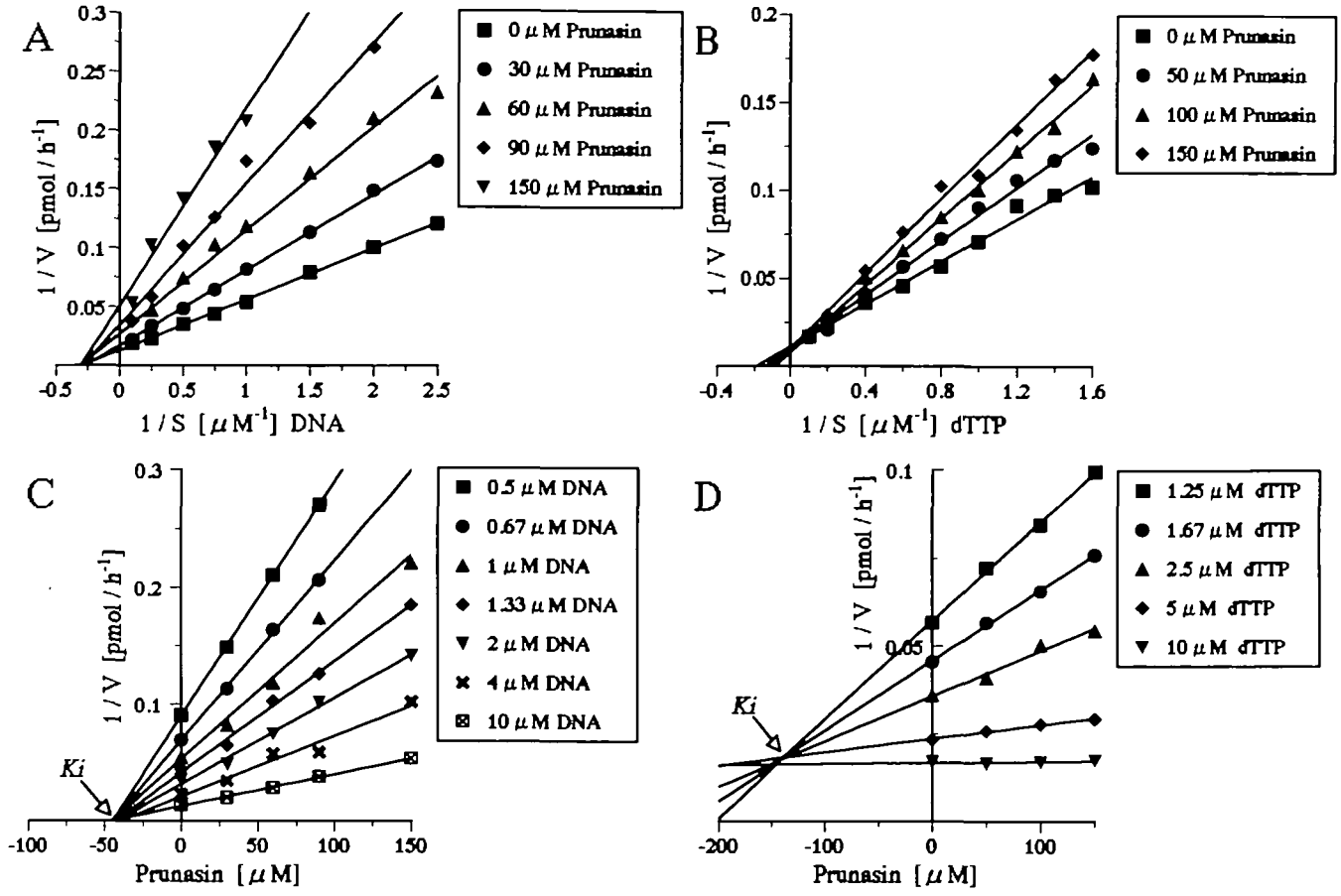

Fig. 3. **Kinetic analysis of the inhibition of pol. β by prunasin with or without capric acid (C_{10}, 10:0).** The effects of mixtures of prunasin and capric acid on the K_m and V_{max} values of the DNA template-primer and nucleotide substrate were determined, and the results are displayed as Lineweaver-Burk plots. (A) Preincubation with pol. β in the presence of 30 μM (●), 60 μM (▲), 90 μM (◆) or 150 μM (▼) prunasin; pol. β activity in the absence of prunasin (■). Pol. β activity was then assayed using the indicated concentrations of poly(dA)/oligo(dT) (2:1) as the template/primer. (B) Pol. β activity was assayed with the concentrations of substrate (dTTP) indicated, after preincubation of pol. β without (■), or with 50 μM (●), 100 μM (▲) or 150 μM (◆) prunasin. (C, D) The inhibition constant (K_i) was obtained at 42 and 140 μM from a Dixon plot on the basis of the data shown in A and B, respectively. (E) Pol. β activity was measured in the absence (■) or presence of 10 μM capric acid (●), 30 μM prunasin and 10 μM capric acid (▲) or 60 μM prunasin and 10 μM capric acid (◆) at the indicated concentrations of the DNA template-primer. (F) The pol. β activity was assayed with the indicated concentrations of substrate (dTTP), after preincubation of pol. β without (■) or with 10 μM capric acid (●), 50 μM prunasin and 10 μM capric acid (▲), or 100 μM prunasin and 10 μM capric acid (◆). (G, H) The inhibition constant (K_i) was obtained at 20 and 5 μM from a Dixon plot on the basis of the data shown in E and F, respectively. Pol. β was added at 0.05 unit.

dTTP was unchanged at 100 pmol/h, a 10-fold increase in the K_m for the substrate, dTTP, was observed in the presence of 100 μM prunasin and 10 μM capric acid (Fig. 3F). The mode of inhibition by the mixture, therefore, was the same as in the case of prunasin alone shown in Fig. 3, A and B, *i.e.* non-competitive or competitive with respect to the DNA template or substrate dTTP. Dixon plots gave K_i values of 20 and 5 μM for the template DNA and substrate dTTP, respectively (Fig. 3, G and H). The K_i values in the presence of 10 μM capric acid were decreased by 2.25- and 28-fold for the template DNA and substrate dTTP, respectively, as compared with those for prunasin alone. Since fatty acids must be present in viable cells, the inhibition by prunasin *in vivo* might be reflected by the observations in the presence of fatty acids. Similar results were observed when oleic acid (C_{18}, 18:1) was used instead of capric acid; inhibition was non-competitive with the template DNA, but competitive with the substrate. For pol. β, the K_m, V_{max}, and K_i with the DNA template were 3.3 μM (unchanged), 80 to 17 pmol/h in the presence of 0 to 40 μM prunasin, and 12 μM, respectively. In contrast, the K_m for the substrate dTTP increased by 10-fold in the presence of 0 to 70 μM prunasin, whereas the V_{max} was unchanged at 100 pmol/h. The K_i was 4 μM for the substrate, dTTP. That is, in the tested concentration range, oleic acid enhanced the effect of prunasin in the same manner as capric acid, although oleic acid at higher levels inhibited polymerase activity through a different mechanism (*15*). As described previously (*15, 23*), fatty acids probably interact with or affect the structural conformation of pol. β, thereby increasing its affinity (*i.e.* decreasing its K_i values) for the DNA template or substrate. Fatty acids bind to the pol. β enzyme, and may cause it to adopt a conformation in which prunasin can more easily gain access to the catalytic site.

Here, we described the characterization of a rat pol. β

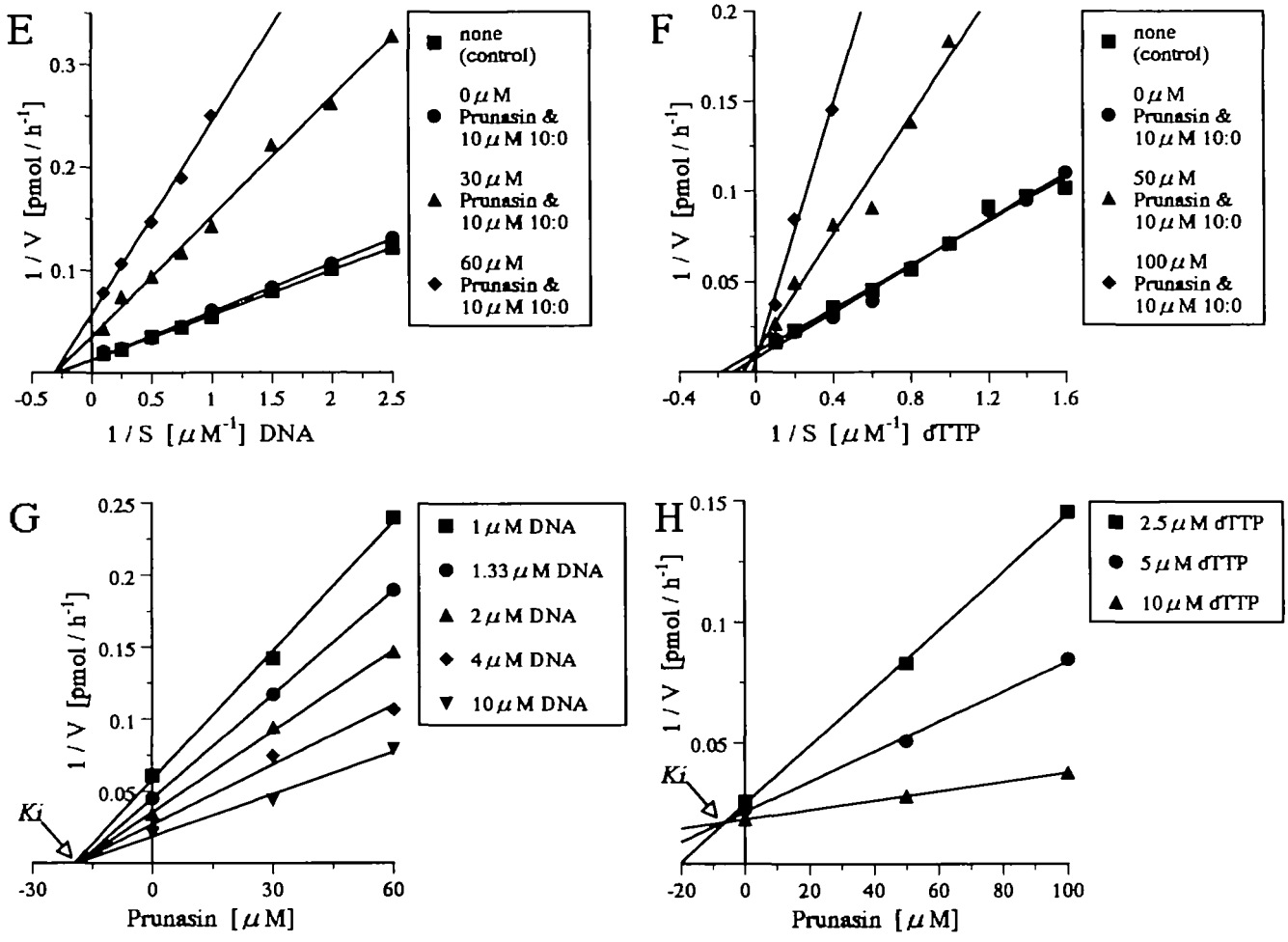

Fig. 3, E-H

inhibitor,which may be specific for pol. β. Whether or not prunasin is a pol. β-specific inhibitor, *i.e.* whether or not it affects other DNA polymerases, should be determined in future studies. The present results showed that prunasin could be a cell-permeable ddTTP. Since such a compound influencing only the pol. β activity and capable of penetrating into viable cells has not been reported previously, prunasin, with fatty acid, could be useful for analyzing both the *in vitro* and *in vivo* functions of pol. β in multicellular organisms. Furthermore, pol. β is expressed and distributed mostly in the thymus and testes, in which the endogenous DNA recombination process occurs (*44*), and subsequently the inhibition of pol. β may influence the meiotic recombination between homologous chromosomes (*9*) and V(D)J recombination in the process of T cell formation (*25, 27*). We speculated previously (*25, 27*) that the immunosuppressive drug, bredinin, is an inhibitor of mammalian DNA polymerases involved in DNA repair and recombination, including pol. β, because these enzymes might be involved to immunological V(D)J recombination. Thus, it is interesting that prunasin is a ddTTP-like agent capable of inhibiting pol. β activity.

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