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Modulation of mutagenicity of various mutagens by lignin derivatives

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Abstract

The effect of lignin on cytotoxicity, mutagenicity and SOS response induced by 4-nitroquinoline-*N*-oxide (4NQO), 3-(5-nitro-2-furyl)acrylic acid (5NFAA), 2-nitrofluorene (2NF) as well as hydrogen peroxide was investigated in bacterial assay systems, i.e. the Ames test with *Salmonella typhimurium* TA98, TA100, TA102 and the SOS chromotest with *Escherichia coli* PQ37. Lignin preparations obtained from beech wood significantly decreased the mutagenicity induced by 4NQO, 2NF and H_2O_2. In the case of mutagenicity induced by 5NFAA the effect was lower. Antimutagenic properties of lignin samples tested were shown also by SOS chromotest where lignin inhibited the ability of both 4NQO and H_2O_2 to induce the SOS response. Derivatives of lignin including those from soft and hard wood, as well as from annual plants differ in their efficiency to inhibit the induction of the SOS response. The modified lignins isolated from beech and spruce wood exhibit a high level of protection. Lignins from annual plants—corn cobs and straw—only marginally evoked an antimutagenic response, but their effect was increased by hydrothermic treatment of both annual plants. The results obtained indicate the prospective utilization of lignin preparations as additive in chemo-prevention. The antimutagenic effect of lignin samples varies with the method of isolation and modification, as well as with the genetic origin of the lignin.

Keywords: Lignin; SOS induction; Antimutagenic effect

1. Introduction

Prevention of cancer and other mutation-related diseases can be pursued by avoiding exposures to recognized mutagens–carcinogens, by fortifying physiological defense mechanisms, or by favoring the intake of protective factors [1]. In view of several drawbacks of synthetic compounds for the human organism, examination of preparations of plant origin for this purpose has received more attention in recent years. Ingredients in dietary and other plants, fruits and seeds may exhibit anticarcinogenic and antimutagenic effects [2,3]. Compounds of plant origin have very diverse structures and functions. Most of the dietary fiber in western diets is in the form of plant cell walls and components derived from these. The available data indicate that plant cell walls containing suberin and lignin may be the most protective, although they are present in only small amounts in food plants [4]. The differences between plant

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components in their antimutagenic properties were revealed by Edenharder et al. [5]. In the microbial system of *Salmonella typhimurium*, mutagenicity of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) was strongly reduced by lignin, weakly by alginic acid and pectin A, while cellulose, gum arabic, gum guar and xylan were ineffective. The potential protective role of lignin biopolymers against carcinogens was also evident from our previous studies including examination of the individual compounds of dietary fiber [6,7]. It has been shown that different lignin preparations exert a binding ability for *N*-nitrosoamines and cholic acids. In contrast with lignin, all tested carbohydrate preparations (microcrystalline cellulose, holocellulose and hemicellulose) were poor adsorbents for *N*-nitrosodiethylamine and bile acids. It was revealed that sulphur-free lignin biopolymer derived from hard-wood hydrolysate reduced DNA strand breaks in H_2O_2- and MNNG-treated mammalian cells [8–10].

Lignin is present in the cell walls of all plants (including edible plants). Its amount in wood and annual plants varies from 17 to 30%. Based on pulp and paper production statistics, about 50×10^6 t per year of technical lignins are produced from woody plants at pulp mills worldwide. For the majority of this material, no better use than burning has been found so far. The anti-immunodeficiency virus activity of natural and synthetic lignin products [11], anti-influenza activity of lignin fractions [12] as well as the above-mentioned anti-genotoxic activity of lignin indicate its potential medicinal applications.

The aim of this work was to examine the antimutagenic effects of lignin towards the mutagens 4NQO, 5NFAA, 2NF and H_2O_2. It is known that the genetic origin and the methods of isolation have a significant effect on lignin properties. For this reason a series of different lignin preparations were tested.

2. Materials and methods

2.1. Chemicals and reagents

o-Nitrophenyl-β-D-galactopyranoside (ONGP) and *p*-nitrophenylphosphate (PNPP) were purchased from Merck, Germany. The positive mutagens 4-nitroquinoline-*N*-oxide (4NQO, Aldrich, USA), 3-(5-nitro-2-furyl)acrylic acid (5NFAA, Slovakofarma, Hlohovec, Slovakia), and 2-nitrofluorene (2NF, Aldrich, USA) were dissolved in DMSO (Merck, Germany). H_2O_2 used was from Lachema Brno, Czech Republic.

2.2. Preparation of lignins

The following lignin samples were especially prepared for the present work, starting from the appropriate materials according to the previously described procedure [13]:

1. Spruce wood kraft lignin.
2. Beech wood kraft lignin.
3. Beech wood pre-hydrolysis lignin.
4. Beech wood condensed pre-hydrolysis lignin.
5. Lignin isolated from alkaline extract of steam-treated wheat straw by precipitation with HCl.
6. Lignin isolated from alkaline extract of steam-treated corn cobs by precipitation with HCl.
7. Lignin isolated from alkaline extract of native straw.
8. Lignin isolated from alkaline extract of native corn cobs.
9. Lignin isolated by acetone extraction of steam-treated wheat straw.

Macromolecular characteristics of lignin derivatives were estimated according to published methods [14,15].

2.3. Bacterial tester strains

Histidin-dependent strains of *S. typhimurium* TA98, TA100 and TA102 were received from the Collection of Microorganisms, Masaryk University, Brno (Czech Republic). They were stored at –80 °C and regularly checked for their genetic markers.

Escherichia coli PQ37 strain was kindly provided by M. Hofnung (Institut Pasteur, Paris, France). The complete genotype, as well as strain construction details can be found in Quillardet and Hofnung [16]. Frozen permanent copies of the tester strain were prepared and stored at –80 °C.

2.4. Genotoxicity assay

Standard plate incorporation assay [17].

For the mutagenicity assay, 0.1 ml of a solution of the tested compound and 0.1 ml of an overnight bacterial culture suspension (cultivation for 16 h at 37 °C, approximate cell density (2–5) $\times$ 10^8 cells/ml) were carefully mixed with 2 ml of melted top agar containing 50 μmol/l of L-histidine–biotin, and poured onto minimal glucose agar plates.

The inhibitory effect of lignin on mutation induction by several positive mutagens was investigated with *S. typhimurium* TA98 using the pre-incubation method. The 0.1 ml of positive mutagen, 0.1 ml of lignin, 0.5 ml of phosphate buffer of pH 7.4 and 0.1 ml of bacterial culture (prepared as in the mutagenicity test) were mixed and preincubated at 37 °C for 30 min. Soft agar (2 ml) was added and poured onto minimal agar plates.

Cell viability was also determined for each anti-mutagenesis experiment to evaluate the potential bactericidal effect. For the determination of surviving cells, a dilution of 1×10^4 was used for the bacterial culture and the mixture was poured onto agar plates with complete medium.

The plates were incubated for 48 h at 37 °C and the number of histidine-independent revertants was counted. Data points represent at least three separate experiments, each run in triplicate. The positive controls were included in each experiment and control plates containing only DMSO, which was used as the solvent vehicle, were also included to obtain the background or spontaneous revertants counts. A positive response for mutagenicity was defined as a reproducible two-fold increase of revertants with a dose–response relationship and statistically evaluated by use of the *t*-test. With the same rationale for a positive anti-mutagenicity response the chemical should reduce the number of revertants induced by the positive mutagen at least by half, this response should be dose-related and the reduction should be statistically significant. Antimutagenicity was expressed as percentage inhibition of the mutagenicity according to the formula $\text{inhibition}\,(\%) = 100 - [(X_1/X_2(100)]$, where X_1 is the number of revertants per plate in the presence of test compound, X_2 the number of revertants per plate in the absence of test compound.

2.5. SOS chromotest

The method of Quillardet and Hofnung [16] was used. An overnight culture of *E. coli* PQ37 (100 μl) was added to 5 ml of fresh La medium and incubated for 2 h at 37 °C. One milliliter of this culture was diluted with 9 ml of nutrient broth. Fractions of 0.6 ml were transferred into a series of glass test tubes, each containing 20 μl of gradual dilutions of the compounds to be tested. The mixtures were incubated with shaking for 2 h at 37 °C. A short centrifugation step followed by elimination of the supernatant and resuspension of the bacterial pellet in buffer was included to avoid problems with the coloration of the lignin solutions and interference with the enzyme assays. After this, two equal sets of tubes were filled with 0.3 ml of each incubated mixture.

To determine the β-galactosidase (β_{gal}) activity induced by DNA-damaging compounds, 2.7 ml of B buffer and 0.6 ml of 0.4% 4-nitrophenyl-β-galactopyranoside (ONGP) solution were added to each tube of one of the sets.

In order to determine the constitutive alkaline phosphatase activity (toxicity assay) P buffer (pH = 8) replaced B buffer, and 0.4% 4-nitrophenylphosphate (PNPP) solution replaced the ONGP solution.

After incubation at 37 °C for 30 min the conversion of ONGP was stopped with 2 ml of 1 M sodium carbonate, and that of PNPP with 2 ml of 1.5 N sodium hydroxide. The absorbance was measured at 420 nm using a blank without bacteria.

For evaluation of the protective effect of lignin on induction of the SOS response by 4NQO, 20 μl of 4NQO solution was added into tubes with 20 μl of tested lignins.

As a measure of genotoxicity, the SOSIP (SOS-inducing potency) was calculated from the linear part of the induction factor dose–response curve. The induction factor (IF) was calculated as the ratio of R_c/R_0, where R_c is equal to β_{gal} activity/AP activity determined for the test compound at concentration c and R_0 is equal to β_{gal} activity/AP activity in the absence of test compound. The β-galactosidase (β_{gal}) and alkaline phosphatase (AP) activities were calculated according to the method recommended by Quillardet and Hofnung [16]. The results are expressed as mean of at least three independent experiments. The statistical significance of calculated values was determined by paired Student's *t*-test.

Table 1
Chemical analysis, molecular mass and cross-linking density of tested lignin derivatives

Sample	N (%)	C (%)	H (%)	OCH_3 (%)	Phenolic OH (%)	Average molecular mass (M_w)	Cross-linking density (ρ)
1	0	64.36	5.80	10.75	4.28	8800	0.0076
2	0	62.95	5.62	22.82	4.07	5248	0.0090
3	0	56.02	5.40	19.10	4.25	2000	0.0095
4	0	56.98	5.80	12.50	4.30	3250	0.0085
5	0.33	54.30	5.93	6.80	2.34	4180	0.0120
6	0.04	60.81	5.75	9.90	2.45	4000	0.0112
7	0.33	54.30	5.39	12.80	2.15	4400	0.0260
8	0.38	55.60	6.15	12.20	2.17	4220	0.0231
9	0.24	68.50	6.66	13.23	5.76	1700	0.0281

1, Spruce wood kraft lignin; 2, beech wood kraft lignin; 3, beech wood pre-hydrolysis lignin; 4, beech wood condensed pre-hydrolysis lignin; 5, lignin isolated from alkaline extract of steam-treated wheat straw by precipitation with HCl; 6, lignin isolated from alkaline extract of steam-treated corn cobs by precipitation with HCl; 7, lignin isolated from alkaline extract of native straw; 8, lignin isolated from alkaline extract of native corn cobs; 9, lignin isolated by acetone extraction of steam-treated wheat straw.

3. Results and discussion

3.1. Analytical data

We have prepared a series of lignin samples from beech and spruce wood as well as from annual plants—wheat straw and corn cobs. The elementary analysis, the molecular mass and cross-linking density of the tested lignins are reported in Table 1.

3.2. Genotoxic activity of lignin

In a series of experiments preceding the anti-mutagenicity studies, it was ascertained that the amount of lignin added to the indicator bacteria does not influence their viability and spontaneous mutation frequencies. The results of Ames test without metabolic activation are in Table 2. The wood lignins (derivatives 1–4) were not toxic to the bacteria at the concentration tested (up to 500 μg/plate), while lignins isolated from corn (derivatives 6 and 8) were toxic to the bacteria at concentrations of 125 and 250 μg/plate and above. Lignins isolated from hydrothermic modified and straw (derivatives 5 and 7) showed toxic effects at the dose of 500 μg/plate.

Lignin preparations 2 and 4 induced a very slight increase of the number of revertants as well as a dose–response. Only a sporadic statistically significant increase was revealed in the case of some lignins. However, in none of the cases did the number of revertants reach the value of twice the control.

The ability of lignin samples to induce an SOS response was also examined. It was revealed that tested lignins in the concentrations used have a very low effect on the induction factor in the SOS chromotest (Table 3), except Sample 6 (IF = 1.56) and Sample 8 (IF = 1.55). According to Kevekordes et al. [18] compounds are classified as non-genotoxic if the induction factor remains <1.5, as marginally genotoxic if the induction factor ranges between 1.5 and 2 and as genotoxic if the induction factor exceeds 2. Based on this, lignins tested are evaluated as non-genotoxic, only the above-mentioned lignins 6 and 8 can be evaluated as marginally genotoxic.

3.3. Anti-mutagenicity studies

Doses of 0.125 μg/plate of 4NQO, 5.0 μg/plate of 5NFAA, 5.0 μg/plate of 2NF were chosen for the anti-mutagenicity studies since these doses were not toxic and induced 636 ± 41 (4NQO), 499 ± 70 (5NFAA) and 896 ± 62 (2NF) revertants in *S. typhimurium* TA98. H_2O_2 in the concentration of 1.87 mg/plate induced 909 ± 69 revertants in *S. typhimurium* TA102. The anti-mutagenicity studies were performed with beech wood pre-hydrolysis lignin (Sample 3). In selecting the concentration of lignin we used the amounts where cell viability was found to be above 90%.

Table 2
Genotoxic activity of lignin derivatives in the Ames test

Lignin	Dose (μg/plate)	TA98		TA100	
		M	S.D.	M	S.D.
1	0	32.0	2.6	219.7	5.0
	31.2	42.6*	6.6	232.3	15.6
	62.5	26.3	5.1	202.3	8.7
	125	28.3	6.0	280.3**	16.5
	250	23.3	4.5	235.3	20.9
	500	31.3	5.8	184.3	13.6
2	0	33.3	1.5	212.7	5.9
	31.2	34.6	3.8	208.3	18.1
	62.5	37.3*	2.3	155.0	9.8
	125	31.3	5.7	254.3**	11.1
	250	32.0	8.9	332.3**	19.8
	500	45.7*	6.0	363.3***	24.5
3	0	34.3	2.3	233.0	8.5
	31.2	36.0	4.6	222.3	14.5
	62.5	38.0	4.0	248.0	21.2
	125	35.6	4.5	241.3	23.2
	250	32.3	3.2	252.3*	12.6
	500	35.0	5.6	212.3	11.5
4	0	31.3	2.1	208.3	7.65
	31.2	31.7	5.5	245.0	16.4
	62.5	38.3	6.7	207.6	14.6
	125	49.7**	6.0	285.3**	13.6
	250	45.3**	3.8	300.3**	22.0
	500	48.3**	6.5	332.3**	19.8
5	0	33.3	3.0	232.3	11.1
	31.2	58.0***	3.0	165.0	17.5
	62.5	57.7**	4.0	235.0	15.3
	125	40.0	2.6	196.3	17.0
	250	55.0**	5.0	244.3	12.8
	500	23.7 (t)[a]	5.1	118.3 (t)[a]	15.2
6	0	35.3	3.8	239.7	19.0
	31.2	45.7*	4.7	450.0***	18.0
	62.5	44.7*	6.0	278.0*	11.7
	125	21.0 (t)[a]	4.4	196.0 (t)[a]	13.4
	250	18.3 (t)[a]	4.0	202.0 (t)[a]	14.0
	500	10.3 (t)[a]	3.5	147.3 (t)[a]	19.3
7	0	32.7	3.5	225.3	15.8
	31.2	31.0	3.4	234.7	8.3
	62.5	42.3*	4.2	255.3**	15.2
	125	37.7	4.0	239.3	16.2
	250	34.7	4.6	231.0	10.1
	500	25.7 (t)[a]	6.5	102.6 (t)[a]	8.5
8	0	34.0	3.0	245.7	13.0
	31.2	53.3**	8.9	284.6*	12.6
	62.5	45.0*	3.0	244.3	13.2
	125	33.0	6.2	255.3	20.0
	250	21.7 (t)[a]	4.1	172.0 (t)[a]	9.5
	500	27.0 (t)[a]	4.0	163.0 (t)[a]	16.5
9	0	31.3	2.1	242.3	7.7
	31.2	39.0	7.0	214.6	12.7
	62.5	31.3	4.0	200.6	18.9
	125	39.0	6.6	229.3	10.0
	250	28.7	4.7	285.6*	15.0
	500	39.23	5.7	175.3	15.5

[a] t: Toxicity.
* $P < 0.1$.
** $P < 0.01$.
*** $P < 0.001$.

Table 3
Genotoxic activity of lignin derivatives in SOS chromotest

Lignin	Dose (μg per assay)	β_{gal}	AP (U)	IF
1	0	4.25	28.63	1.00
	1.04	3.84	26.67	0.97
	2.08	2.83	25.67	0.74
	4.16	4.50	27.50	1.10
	8.33	4.25	23.50	1.22
	16.66	nd	nd	nd
2	0	3.86	29.14	1.00
	1.04	3.83	28.33	1.00
	2.08	4.33	26.79	1.20
	4.16	3.50	25.83	1.01
	8.33	4.08	24.58	1.24
	16.66	nd	nd	nd
3	0	2.07	27.49	1.00
	1.04	1.05	27.11	0.51
	2.08	2.25	23.33	1.28
	4.16	1.83	25.16	0.97
	8.33	1.67	20.00	1.11
	16.66	nd	nd	nd
4	0	3.83	30.58	1.00
	1.04	4.67	30.00	1.24
	2.08	4.08	28.33	1.15
	4.16	4.25	27.36	1.24
	8.33	4.92	33.17	1.18
	16.66	nd	nd	nd
5	0	3.81	28.45	1.00
	1.04	3.75	25.00	1.12
	2.08	3.86	28.36	1.02
	4.16	4.00	27.50	1.09
	8.33	3.67	24.78	1.11
	16.66	nd	nd	nd
6	0	3.92	31.67	1
	1.04	4.67	29.42	1.28

Table 3 (*Continued*)

Lignin	Dose (μg per assay)	β_{gal}	AP (U)	IF
	2.08	5.08	28.33	1.45
	4.16	6.42	33.17	1.56
	8.33	5.47	33.11	1.42
	16.66	nd	nd	nd
7	0	2.56	28.46	1.00
	1.04	2.62	26.66	1.09
	2.08	3.12	27.19	1.28
	4.16	2.71	28.77	1.05
	8.33	1.59	24.12	0.73
	16.66	nd	nd	nd
8	0	3.99	32.55	1.00
	1.04	4.42	28.12	1.28
	2.08	6.33	33.33	1.55
	4.16	5.67	33.27	1.39
	8.33	4.83	29.16	1.35
	16.66	nd	nd	nd
9	0	4.13	34.53	1.00
	1.04	3.83	27.50	1.13
	2.08	4.50	26.85	1.36
	4.16	4.75	28.33	1.36
	8.33	4.75	29.17	1.32
	16.66	nd	nd	nd

nd, not determined due to low activity of alkaline phosphatase.

The inhibitory effect of the lignin on the mutagenicity of positive mutagens using the plate incorporation assay is illustrated in Table 4. The addition of the lignin sample reduced the 4NQO-induced mutagenesis and a clear dose–response effect was noticed. In the concentrations of 125, 250 and 500 μg/plate, the lignin sample reduced mutagenesis by 37, 57 and 85%, respectively.

The mutagenic activity of 5NFAA was not affected by the lignin sample tested, only sporadic decreases of the number of revertants were recorded with some of the concentrations.

The number of revertants induced by 2-nitrofluorene was statistically significantly reduced to about 55% by lignin preparation at concentration of 62.5 μg/plate and the use of higher concentrations of lignin resulted in a further significant decrease of the number of revertants compared with the control. The highest concentration of 500 μg/plate resulted in a decrease of 85% in the number of revertants.

The strain *S. typhimurium* TA102 was used for the determination of lignin's influence on the number of revertants induced by H_2O_2, because this strain has been shown to be highly sensitive to reactive oxygen species [19]. The effect of H_2O_2 was significantly reduced and within the concentrations tested a marked dose-dependence was found.

Table 4
Effect of beech wood pre-hydrolysis lignin on mutagenicity induced by 4NQO, 5NFAA, 2NF and H_2O_2

	Concentration of lignin (μg/plate)	TA98		TA102	
		M	S.D.	M	S.D.
4NQO	0	696	41	–	–
	31.2	688	61	–	–
	62.5	660	25	–	–
	125	440**	55	–	–
	250	298***	42	–	–
	500	108***	16	–	–
5NFAA	0	499	57	–	–
	31.2	409	67	–	–
	62.5	444	47	–	–
	125	364*	45	–	–
	250	434	96	–	–
	500	213**	48	–	–
2NF	0	896	62	–	–
	31.2	800	59	–	–
	62.5	475**	68	–	–
	125	242***	35	–	–
	250	334***	42	–	–
	500	139***	42	–	–
H_2O_2	0	–	–	909	69
	31.2	–	–	799*	55
	62.5	–	–	766*	43
	125	–	–	737**	51
	250	–	–	463***	43
	500	–	–	316***	34

* $P < 0.1$.
** $P < 0.01$.
*** $P < 0.001$.

Induction of SOS response in *E. coli* PQ37 by 4NQO as well as by H_2O_2 was also affected by addition of lignin. The SOSIPs of 4NQO as well as H_2O_2 were determined at various concentrations of beech wood pre-hydrolysis lignin (Sample 3) and it was revealed that the induction of SOS response significantly decreased as a function of lignin concentration (Figs. 1 and 2). With lignin at a concentration of 8.33 μg per assay, the SOSIP of 4NQO was decreased about 64% and, at the same concentration of lignin, the SOSIP of H_2O_2 was decreased about 57%.

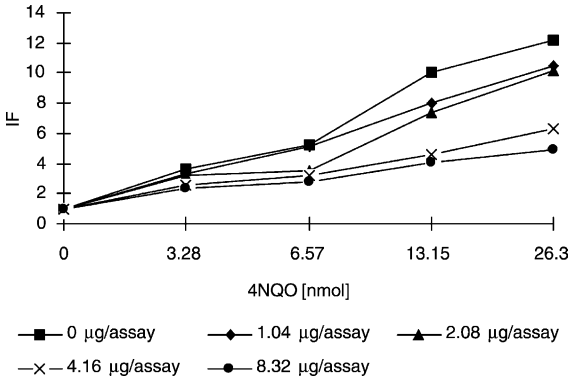

Fig. 1. The effect of concentration of beech wood pre-hydrolysis lignin (Sample 3) on the induction factor of 4NQO. The data are the mean from at least three independent experiments.

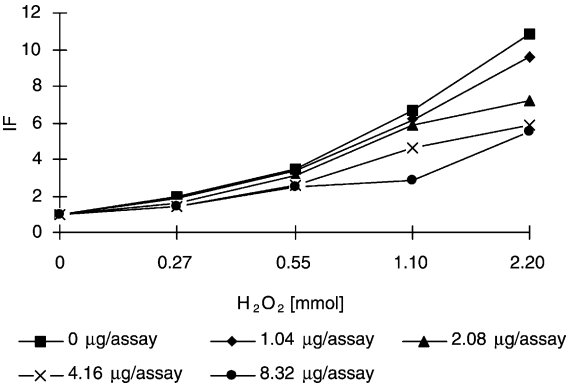

Fig. 2. The effect of concentration of beech wood pre-hydrolysis lignin (Sample 3) on the induction factor of H_2O_2. The data are the mean from at least three independent experiments.

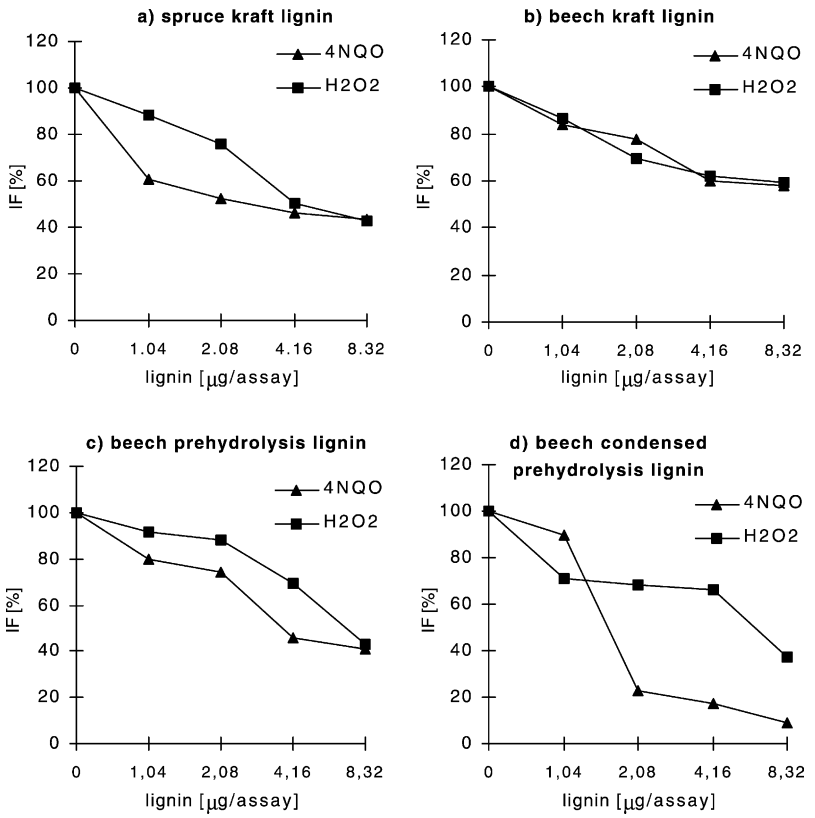

Fig. 3. Effect of wood-derived lignins on 4NQO (13.15 nmol) and H_2O_2 (1.1 mmol) induced SOS response. The data are the mean from at least three independent experiments.

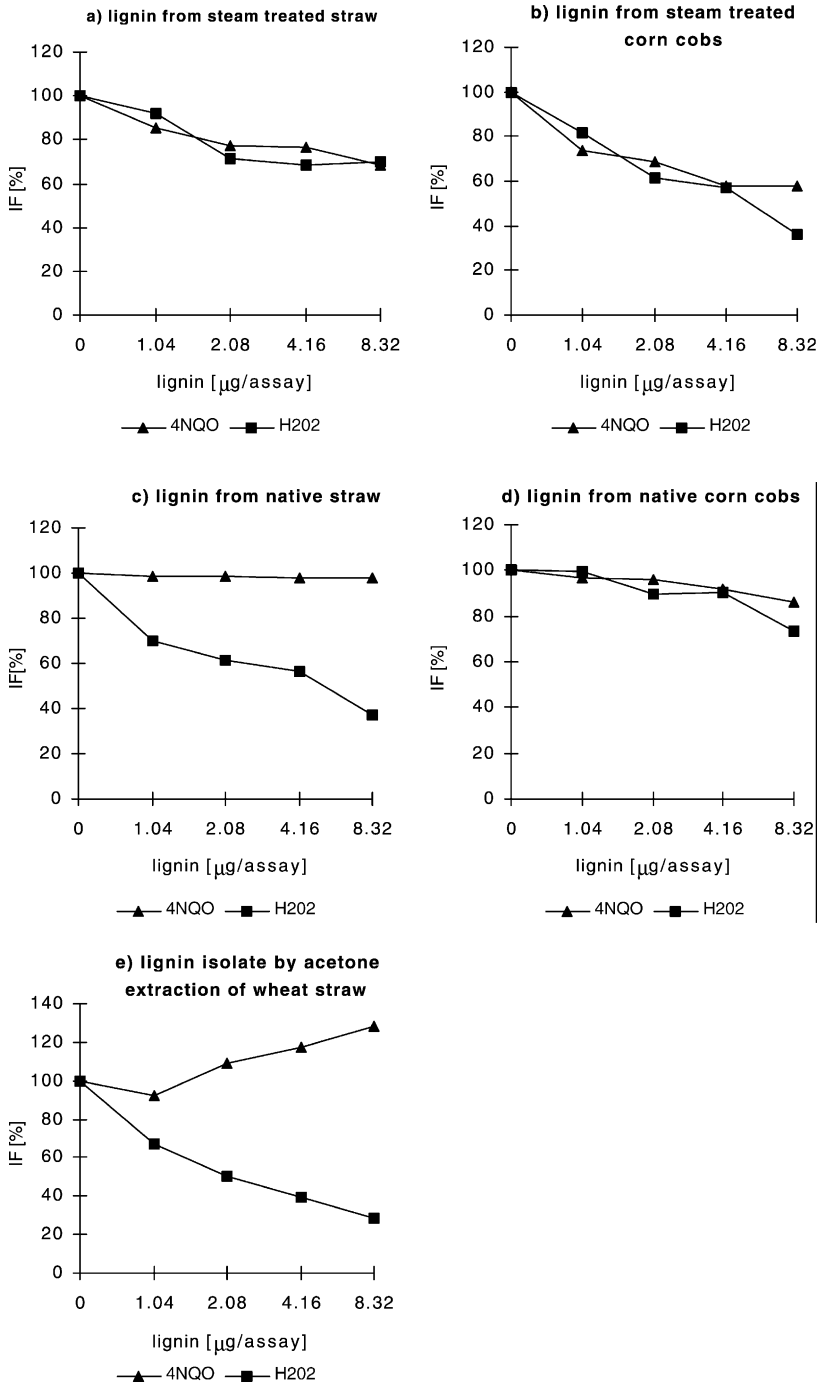

Fig. 4. Effect of lignins isolated from wheat straw and corn cobs on SOS response induced by 4NQO (13.15 nmol) and H_2O_2 (1.1 mmol). The data are the mean from at least three independent experiments.

3.4. The effect of lignin derivatives on the induction of SOS response by 4NQO and H_2O_2

These experiments were performed in order to examine to which extent antimutagenic properties of lignin are influenced by its genetic origin and methods of isolation.

As shown in Fig. 3, all modified wood lignins were effective in reducing the IF induced by 4NQO as well as by H_2O_2. Kraft lignin derivatives 1 and 2 isolated from spruce and beech wood exhibited a similar efficiency (Fig. 3a and b). Sample 3 derived from beech wood pre-hydrolysate has an effect comparable with that of both kraft lignins (Fig. 3c). The modification of Sample 3 yielded lignin preparation 4, which exhibited the strongest ability to decrease activity of 4NQO (Fig. 3d). The determination of molecular parameters of lignins 3 and 4 shows that the modification increased the average molecular weight about 62.5% and decreased cross-links about 10.5% (Table 1). The increased efficiency of lignin 4 could be explained by degradation of cross-links and linearization of the lignin macromolecule during modification [7].

The samples prepared from steam-treated annual plants show a relatively lower efficiency than those isolated from wood.

The lignin preparations isolated from the alkaline extracts of steamed straw and corn cobs (Fig. 4a and b) were more effective antimutagens than those isolated from native straw and corn cobs (Fig. 4c and d). Generally, it is known that lignin from annual plants is a copolymer of *p*-hydroxyphenyl, quaiacyl and syringyl units, approximately in the ratio of 1:1:1. The hydrolysis of wheat and corn lignin during hydrothermal treatment is accompanied mainly by condensation of *p*-hydroxyphenyl units with free 3- and 5-positions in the aromatic ring. The higher activity of lignins isolated from steamed samples can be explained in terms of condensation reactions of lignin during the steaming process. From the comparison of lignin preparations from straw and corn cobs it is clear that the latter decrease the mutagenicity of 4NQO more effectively.

Low molecular weight lignin Sample 9 obtained by acetone extraction of steamed wheat straw shows no antimutagenic properties and it significantly increased IF of 4NQO, e.g. its mutagenic effect. In contrast, the sample decreased the IF of H_2O_2 probably due to its high content of phenolic hydroxyl groups (Fig. 4e).

The results obtained in this study indicate that tested lignin derivatives decrease mutagenic activity of 4NQO, 2NF and H_2O_2. The observed differences in lignin efficiency could be explained in terms of the genetic origin and methods of modification of the lignin.

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