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The Effect of 1,2-Naphthoquinone on Catechol 1,2-Oxygenase from *Trichosporon cutaneum*

JANOS M. VARGA* and HALINA Y. NEUJAHR

Department of Pure and Applied Biochemistry, The Royal Institute of Technology, S-100 44 Stockholm 70, Sweden

Catechol 1,2-oxygenase purified from *Trichosporon cutaneum* is reversibly inhibited by micromolar concentrations of 1,2-naphthoquinone (NQ). Freshly prepared crude enzyme is less sensitive to this inhibition than purified preparations.

The crude enzyme is activated 2 – 3 fold upon storage. This causes an apparent increase (2 – 9 fold) of the total enzyme yield during purification. The storage-activated crude enzyme is as sensitive to NQ as is the purified one. The presence of an endogenous inhibitor with *o*-quinone structure is suggested.

We have recently reported that a significant increase in the total yield of catechol 1,2-oxygenase (EC 1.13.1.1.) occurred during purification of the enzyme from *Trichosporon cutaneum*.[1] Furthermore, the crude or protamine sulphate treated enzyme, but not the purified one, became activated upon storage or after dialysis. These observations indicated the occurrence of an endogenous inhibitor.

Endogenous inhibitors have been demonstrated in the case of several other oxygenases. With some monooxygenases these were identified as thiol compounds.[2] However, a number of investigated thiol compounds (2-mercapto-ethanol, 2-mercapto-ethylamine, cysteine, and reduced glutathione) did not inhibit our enzyme. We were, therefore, looking for other possible candidates among the inhibitors of the purified enzyme. This led to the investigation of the effect of *o*-quinones, which we have earlier found to act as potent inhibitors of catechol 1,2-oxygenase purified from *T. cutaneum*. In this paper we report on the effect of 1,2-naphthoquinone on catechol 1,2-oxygenase prepared from *T. cutaneum*. Preliminary results were reported earlier.[3]

MATERIALS AND METHODS

Commercially available chemicals of reagent grade were used. 1,2-naphthoquinone was a product of Schuchardt (München, West Germany) and was used without further

* Present address: Department of Internal Medicine, Yale Medical School, 333 Cedar Street, New Haven, Conn. 06510, USA.

purification, immediately after dissolution. *o*-Benzoquinone was synthesized by the method of Willstätter and Pfannenstiel.[4]

The details of the purification of catechol 1,2-oxygenase from *T. cutaneum* and the spectrophotometric assay of enzyme activity were as described elsewhere.[2] Ultrafiltration was carried out in a Diaflo Model 50 Ultrafiltration cell (Amicon Co., Lexington, Mass.), with a membrane No. XM 50, which retained the enzyme.

RESULTS AND DISCUSSION

Fig. 1 shows progress curves for our purified enzyme in the presence of different concentrations of naphthoquinone. It can be seen that already micromolar concentrations of the quinone caused a significant inhibition of the enzyme. At quinone concentrations higher than 10 mM the progress curves were not linear and the enzyme appeared partly released of inhibition after the first $1-2$ min.

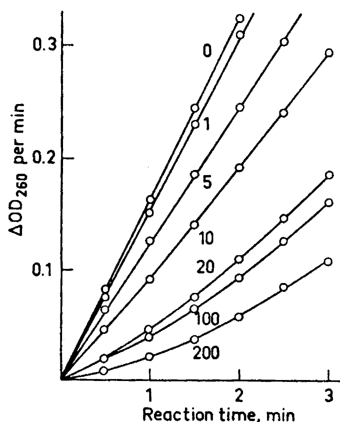


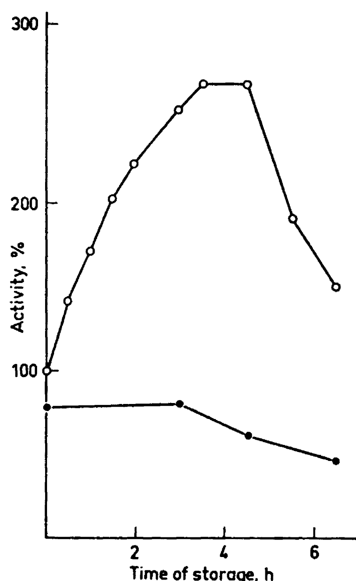
Fig. 1. Inhibition of purified catechol 1,2-oxygenase by 1,2-naphthoquinone. The reaction mixture contained in 3 ml: 100 μmol Tris-Cl pH 8.3, 10 μmol 2-mercaptoethanol, 0.2 μmol catechol, 0.02 mg enzyme protein, purified by treatment with protamine sulphate, ammonium sulphate precipitation and chromatography on DEAE-Sephadex (step 4 of Ref. 1). The reaction was initiated by the addition of catechol. Concentrations of 1,2-naphthoquinone, μmoles as indicated for the individual progress curves. The reaction rate was measured by following the increase in absorbance at 260 nm. Readings were taken at 20 sec intervals for 3 min at room temperature.

The order of addition of the reaction components was of importance; the inhibitor had to be added before the addition of the substrate. When the inhibitor was added after the reaction was initiated with the substrate, the enzyme was markedly less sensitive to inhibition.

The activation of a crude enzyme preparation upon storage at 31°C is demonstrated in Fig. 2. The activity of the enzyme increases for about 3 1/2 h, whereafter it gradually decreases. However, when the enzyme assay was carried out in the presence of 0.1 mM naphthoquinone, the activity of the enzyme remained practically unchanged for 2 1/2 h.

Fig. 3 shows the effect of increasing concentrations of naphthoquinone on freshly prepared enzyme before and after storage at 31°C. It is seen that a 50 % inhibition of the crude enzyme required about 100 times higher concentrations of naphthoquinone as compared to the enzyme which had been activated by storage.

These results indicated that the activation of the crude enzyme upon storage and the increase of the enzyme yield during the purification process may be a result of the elimination of an *o*-quinone derivative present in the



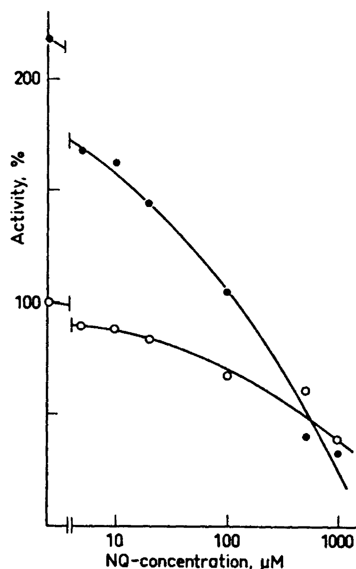


Fig. 2. Activation of a crude preparation of catechol 1,2-oxygenase upon storage at 31°C and the effect of 1,2-naphthoquinone on the stored enzyme. Assay as in Fig. 1 using freshly prepared, protamine sulphate treated enzyme, (step 2 of Ref. 1), 0.15 mg protein. The enzyme was stored in a water bath at 31°C, aliquots were withdrawn after the time intervals indicated and the enzyme activity was measured in the absence (○) and in the presence (●) of 0.1 mM 1,2-naphthoquinone.

Fig. 3. Inhibition of fresh and storage-activated catechol 1,2-oxygenase by 1,2-naphthoquinone. Assay as in Fig. 1 using enzyme as in Fig. 2. Concentrations of 1,2-naphthoquinone as indicated on the abscissa. ○, enzyme assay before storage; ●, enzyme assay after storage at 31°C for 3 h.

Table 1. The reversibility of inhibition by 1,2-naphthoquinone of protamine-treated, activated catechol 1,2-oxygenase. A solution of the enzyme, 3.0 mg protein/ml in 10.0 ml was made up to contain 0.1 mM naphthoquinone. Subsequently, the concentration of the inhibitor was reduced by repeated ultrafiltration and dilution to values below 0.0001 mM. Diaflo XM-50 membrane was used. Enzyme activity was determined without inhibitor, after the addition of the inhibitor and after ultrafiltration. Assay and enzyme as in Fig. 1.

Conditions of assay	Enzyme activity	
	$\Delta OD_{260/min}$	%
1. No inhibitor	0.195	100
2. +0.1 mM 1,2-naphthoquinone	0.050	26
3. Inhibitor removed by ultrafiltration	0.185	95

freshly prepared crude extract. We have therefore studied the reversibility of the inhibition. Table 1 shows the results of experiments, in which the inhibitor was removed by ultrafiltration. It is seen that the activity of the enzyme which had been inhibited by naphthoquinone to 26 % of the original, can be almost completely restored when the inhibitor is removed.

In the experiments described above we have employed *o*-naphthoquinone as a model of an *o*-quinone inhibitor. This compound is not likely, however, to occur in our enzyme system. Rather, one would expect the occurrence of *o*-benzoquinone, which can be formed from catechol by enzymatic or non-enzymatic oxidation. At very high concentrations, *o*-benzoquinone inhibited the enzyme. However, at concentrations below 1 mM the inhibition was almost insignificant and there was no difference between the sensitivity of the freshly prepared and the storage activated enzyme. Results obtained with *o*-benzoquinone may be misleading, however, because of the extreme instability of this compound in water solutions. One can also envisage a rapid reduction of the quinone in the presence of mercapto-ethanol, which was employed in our assay of catechol 1,2-oxygenase.[2] The data obtained with *o*-benzoquinone are therefore not reported.

The results presented here indicate that catechol 1,2-oxygenase in *Trichosporon cutaneum* has an endogenous inhibitor, which is probably an *o*-quinone derivative. We suppose that NQ is an analogue of this endogenous inhibitor. The inhibitor may have a structure similar to that reported for the inhibitor of mitochondrial respiration isolated from mushroom by Weaver *et al.*[5] and identified as γ-L-glutaminyl 3,4-benzoquinone.[5] This inhibitor is reported to react very rapidly, completely, and at low concentration with the sulfhydryl groups of various mitochondrial enzymes. In certain cases the inhibition is reported to take place in the presence of O_2, but not in the presence of a redox dye. It may thus be anticipated that the inhibitor interferes with the very mechanism of O_2 uptake. This has a particular bearing on our observation of the inhibition of catechol 1,2-oxygenase by naphthoquinone and possibly by benzoquinone.

The activation of our enzyme upon storage and the increase of the total enzyme yield during enzyme purification is a consequence of the destruction or elimination of the endogenous inhibitor. Since we have observed 2 – 9 fold activation of the crude enzyme upon storage or purification, the enzyme in the cell may be inhibited up to 90 %. This may indicate some regulatory role of the endogenous inhibitor.

REFERENCES

1. Varga, J. M. and Neujahr, H. Y. *Eur. J. Biochem.* **12** (1970) 427.
2. Nagatsa, T. In Bloch, K. and Hayaishi, O., Eds., *Biological and Chemical Aspects of Oxygenases*, Maruzen, Tokyo 1966, p. 273.
3. Varga, J. M. and Neujahr, H. Y. *Fed. Eur. Biochem. Soc. Proc. Meet., Abstr.* **6** (1969) No. 181.
4. Willstätter, R. and Pfannenstiel, A. *Ber.* **37** (1904) 301.
5. Weaver, R. F., Rajagopalan, K. V., Handler, P., Jeffs, P., Byrne, W. L. and Rosenthal, D. *Proc. Natl. Acad. Sci. US* **67** (1970) 1050.

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