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Purification and Properties of Quinone Reductase

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Abstract

Quinone reductase was purified to electrophoretic homogeneity from bovine liver by using ammonium sulfate fractionation, ion-exchange chromatography, and gel filtration chromatography. The enzyme utilized either NADH or NADPH as the electron donor. The optimum pH of the enzyme was pH 8.5, and the activity of the enzyme was greatly inhibited by Cu^{2+} and Hg^{2+} ions, dicumarol and cibacron blue 3GA. The enzyme catalyzed the reduction of several quinones and other artificial electron acceptors. Furthermore, the enzyme catalyzed NAD(P)H-dependent reduction of azobenzene or 4-nitroso-N,N-dimethylaniline. The apparent K_m for 1,4-benzoquinone, azobenzene, and 4-nitroso-N,N-dimethylaniline was 1.64mM, 0.524mM and 0.225mM, respectively. The reduction of azobenzene or 4-nitroso-N,N-dimethylaniline by quinone reductase was strongly inhibited by dicumarol or cibacron blue 3GA, potent inhibitors of quinone reductase.

Introduction

Quinone reductase, also referred to as DT-diaphorase, catalyzes the two-electron reduction of quinones to hydroquinones. Quinone reductase is known to be a xenobiotic metabolizing enzyme and is highly inducible in animals following pretreatment with various xenobiotic chemicals including polycyclic aromatic hydrocarbons and other planar aromatic compounds. Quinone reductase was purified from bovine liver and some of its properties were investigated.

Materials and Methods

Quinone reductase activity was measured in 25mM Tris-HCl buffer(pH 8.0) containing 100μM 1,4-benzoquinone and 200μM NAD(P)H. Protein concentration

was determined according to the method of Bradford. Quinone reductase was purified to apparent homogeneity by a combination of ammonium sulfate fractionation, ion-exchange and gel permeation chromatographies. SDS-polyacrylamide gel electrophoresis was performed according to the procedure described by Laemmli.

Results and Discussion

Table 1. Purification of quinone reductase from bovine liver.

Purification step	Total protein (mg)	Total activity (U)[a]	Specific activity (mU/mg)	Purification (fold)	Recovery (%)
Crude extract	2885	0.8355	0.2896	1	100
Ammonium sulfate fractionation	518.9	0.4077	0.7857	2.71	48.8
CM-Sepharose CL-6B	30.64	0.4007	13.08	45.17	47.9
Sephacryl S-200-HR	3.406	0.3144	92.31	318.8	37.6

[a]1U = 1 μmol of NADH oxidized min^{-1}

Table 2. Substrate specificity of the purified quinone reductase.

Substrate (100 μM)	Relative enzyme activity (% of control)	
	NADH (200 μM)	NADPH (200 μM))
1,4-Benzoquinone	100	72.2
2,6-Dichlorophenolindophenol	88.6	177
2,6-Dimethylbezoquinone	121.1	96.6
2-Methyl-1,4-naphthoquinone	28.3	74.3
Azobenzene	35	10.2
4-Nitroso-N,N-dimethyaniline	25.4	82.0

The reaction mixture consisted of 100 μM of the indicated substrate, 200 μM NAD(P)H, 100mM sodium phosphate buffer(pH 7.0) and the enzyme. Reaction rates are expressed relative to the rate with 1,4-benzoquinone(100 μM) and NADH(200 μM) as equal to 100.

Table 3. Kinetic constants for the purified quinone reductase.

Substrate	Vmax (μmol/min/mg)	K_m (mM)	V_{max}/K_m
1,4-Benzoquinone	9.69	1.64	5.91
Azobenzene	0.218	0.524	0.416
4-Nitroso-N,N-dimethylaniline	0.150	0.225	0.667

A fixed NADH concentration of 200 μM was used in determining the K_m for the electron acceptors. The reaction mixture contained 100mM sodium phosphate buffer(pH 7.0), 200 μM NADH, purified quinone reductase and varied concentrations of 1,4-benzoquinone, azobezene or 4-nitroso-N,N-dimethyaniline.

References

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