Abstract

The system involved in the reduction of 2-[4'-di(2n-bromopropyl)aminophenylazo]benzoic acid (CB10-252), an agent designed for treating primary liver cell cancer, has been demonstrated to be localised mainly in the 108 000 × *g* supernatant fraction of rat liver homogenate. It is also present in other organs particularly in the spleen. DAB-azoreductase as shown previously is present almost entirely in the microsomal fraction and is found in high concentration only in liver. The pH maxima for CB10-252-azoreductase and DAB-azoreductase were 6.2 and 6.9, respectively. Methylred-azoreductase had properties in most respects similar to CBlO-252-azoreductase implying the importance of the 2'-car□yl group in determining substrate specificity.

The use of enzyme inhibitors and other additives showed that CB10-252 was not xanthine oxidase or dihydrofolate reductase. Its activity was not affected by carbon monoxide, phenobarbitone (PB), or 3-methylcholanthrene (MC) pretreatment. Enhancement of the activity by ferrous ions and FAD indicated that at least part of the reduction system could involve a flavoprotein with FAD as the prosthetic group.

The activity of CB10-252-azoreductase and methylred-azoreductase was reduced by menadione (vitamin K_3), cyanide and propylgallate.

A diaphorase preparation from pig heart reduced both CB10-252 and methylred with both NADPH- and NADH-generating systems.

The properties listed above and dependency of enzyme activity on both NADH and NADPH indicate a similarity of CB-10-252 and methylred-azoreductases to DT-diaphorase (NAD(P)H dehydrogenase).

Non-enzymatic reduction of CB10-252 was achieved by both glutathione and ascorbic acid in high concentration.

The involvement of different enzyme systems in the reduction of DAB on the one hand and CB10-252 and methylred (MeRed) on the other, are probably due largely to the presence of the 2'-car□ylic group and its ability to form a hydrogen bond with the б-nitrogen atom of the azo linkage.

Abbreviations

- CB10-252, 2-[4'-di(2n-bromopropyl)aminophenylazolbenzoic acid;
- pCMB, *p*-chloromercuric benzoate;
- DAB, *N,N*-dimethyl-4-phenylazoaniline;
- DMSO, dimethylsulfoxide;
- Lilly 008-163-163, 2,4-dichloro-6-phenylphenoxy-ethyl diethylamine hydrobromide;
- MC, 3-methylcholanthrene;

- MeRed, 2-(4'-*N,N*-dimethylaminophenylazo)benzoic acid;
- PB, phenobarbitone;
- SKF 525-A, 2-diethylaminoethyl-2,2-diphenyl valerate hydrochloride;
- TCA, trichloroacetic acid