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PROTECTION BY GSH AGAINST LIPID PEROXIDATION INDUCED BY ASCORBATE AND IRON IN RAT LIVER MICROSOMES

Raymond F. Burk
Gastroenterology Division, Department of Medicine
University of Texas Health Science Center
7703 Floyd Curl Drive
San Antonio, Texas 78284 U.S.A.

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GSH is considered to be a potent inhibitor of lipid peroxidation, but the mechanisms by which it carries out this function are not clear. GSH-dependent factors which inhibit lipid peroxidation in the NADPH and in the ascorbate-iron microsomal lipid peroxidation systems have been demonstrated in rat liver 105,000 g supernatant (1,2). This communication describes a GSH-dependent factor in the microsomal fraction of rat liver which inhibits ascorbate and iron-induced microsomal lipid peroxidation.

METHODS

Microsomes were isolated as previously described (2) from rats fed a nutritionally adequate semipurified diet containing corn oil as the fat source (3). The incubations were carried out in a shaking water bath at 37°C under air. The incubation buffer was 50 mM Tris HCl, pH 7.5 containing 0.14 M NaCl. The volume of each incubation was 5 ml and final concentrations were: microsomal protein 0.5 mg/ml, ascorbate 0.5 mM, ADP 2 mM, $FeCl_3$ 6 μM. The ADP and iron were mixed and left at room temperature for an hour before use. TBA-reactive substances and protein were assayed as described in reference 2.

RESULTS

Figure 1 demonstrates the effect of GSH in the ascorbate-iron microsomal lipid peroxidation system. In the absence of GSH, a 1-2 minute lag (lag I) was seen before lipid peroxidation products were detected. The presence of 0.1 mM GSH in the flask lengthened the lag by over 4 minutes (lag II). In various experiments lag I varied but was always 4 minutes or less. Lag II also varied and was occasionally as long as 20 minutes. Lag II was abolished by heating the microsomes in boiling water for 1 minute (not shown), suggesting the involvement of a protein in the GSH effect. Extensive washing of the microsomes did not eliminate lag II (not shown), suggesting that the factor is bound to the microsomal membrane.

DISCUSSION

These observations indicate that there is a GSH-dependent microsomal factor, probably protein in nature, which is capable of inhibiting microsomal lipid peroxidation. Such a factor could be crucial in protecting the highly-unsaturated fatty acids of the microsomal membrane from free radicals generated by the function of the cytochrome P-450 system. More studies will be required to characterize this factor and to determine its mechanism of action.

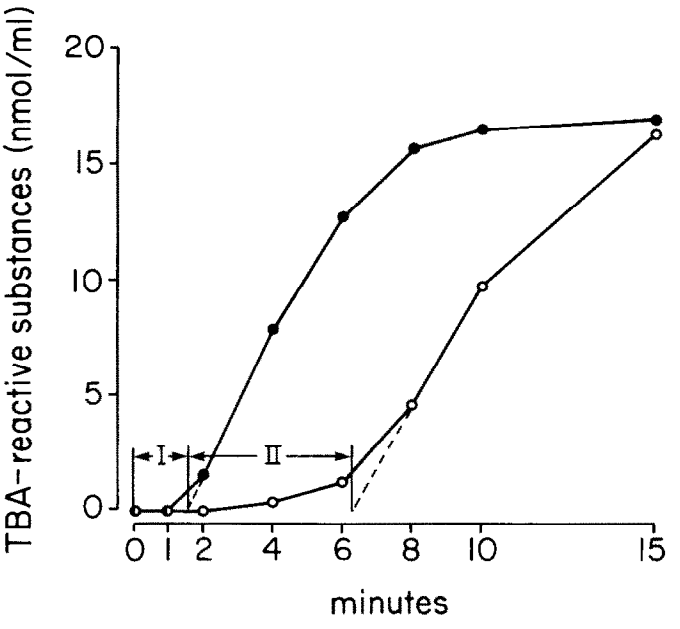

Figure 1. 'Effect of GSH on ascorbate-iron microsomal lipid peroxidation. Closed circles denote no GSH in the flask. Open circles denote 0.1 mM GSH in the flask. Incubation conditions are given in METHODS.

ACKNOWLEDGEMENTS

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