that our method is working and we believe that the transposon induced mutants in the RNA methylation will facilitate the study of the biosynthesis and function of this group of modifying enzymes and contribute to a more thorough understanding of the translational apparatus in *E. coli*.

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Modification of the Binding Site for Pyridine Nucleotides of Glutathione Reductase by 2,3-Butanedione*

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Glutathione reductase has two different binding sites per subunit: one, N-site, for the pyridine nucleotide substrate (NADPH**) and one, G-site, for the disulfide substrate (GSSG).[1,2] The two sites, whose openings are located on opposite sides of the same subunit, communicate *via* an isoalloxazine ring of FAD at the bottom of the N-site and a juxtaposed cystine disulfide at the bottom of the G-site. Thus, reducing equivalents are transferred from NADPH in the N-site to GSSG in the G-site *via* two redox-active groups in the interior of the enzyme. Site-specific inhibitors could help to unravel the catalytic events and locate them topographically in the enzyme molecule. Two such inhibitors have recently been found, which affect the G-site: ethoxyformic anhydride which modifies an essential histidine[3] and 2,4,6-trinitrobenzenesulfonate which appears to interact with the reduced form (dithiol) of the redox-active disulfide.[4] However, no inhibitor modifying the N-site has been reported. The widespread occurrence of arginine in anion-binding sites[5] prompted the present study, which shows that the arginine-modifying reagents 2,3-butanedione and phenylglyoxal inhibit the enzyme by reacting with the N-site.

Glutathione reductase from human erythrocytes (*cf.* Refs. 3 and 6 for experimental procedures) was irreversibly inhibited by 2,3-butanedione in 50 mM sodium borate (pH 8.3). The inhibition increased with time and with the concentration of 2,3-butanedione, and was strongly dependent on the presence of borate, as expected for arginine modification.[5] The absorption spectrum of the enzyme was not affected in the region 350 – 600 nm by the modification. The enzyme was similarly inactivated by phenylglyoxal. Fig. 1 shows the progress curves of the inactivation with 2,3-butanedione in the absence and presence of NADPH. It was found that 2 mM NADPH

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** Abbreviations: FAD, flavin adenine dinucleotide; EDTA, ethylenediaminetetraacetate; GSSG, glutathione disulfide; NADP+ and NADPH, oxidized and reduced forms of nicotinamide adenine dinucleotide phosphate.

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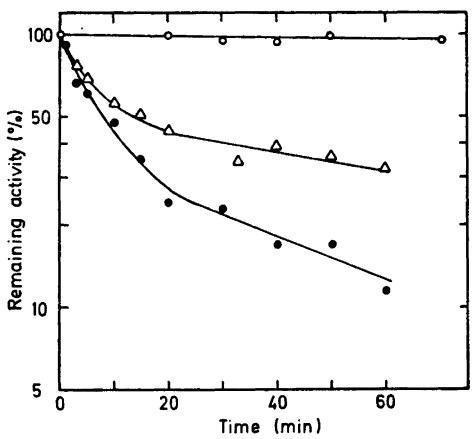

Fig. 1. Progress curves of the inactivation of glutathione reductase from human erythrocytes with 10 mM 2,3-butanedione in absence (●) or presence (△) of 2 mM NADPH. The enzyme was preincubated with the reagent in 50 mM sodium borate (pH 8.3) containing 1 mM EDTA and the residual GSSG-reducing activity was measured in aliquots transferred to a standard assay system (*cf.* Ref. 6). The control (○) was incubated in the absence of 2,3-butanedione. Essentially the same results were obtained when the transhydrogenase activity of the N-site was assayed by using NADPH and thionicotinamide-adenine dinucleaotide phosphate as substrates.

protected the enzyme partially and that the following analogs of NADPH had similar protective effects (at 5 mM concentration): $NADP^+$, 2′,5′-adenosine diphosphate, 2′- and 3′-adenosine monophosphate, and inorganic pyrophosphate (in the order of decreasing effectiveness). GSSG could not be shown to protect the enzyme significantly. These findings indicate that the inhibition is directed *versus* the N-site. The transhydrogenase reaction catalyzed by the N-site was inhibited to the same extent as the GSSG reduction (*ca.* 80 % inhibition after exposure for 60 min to 10 mM 2,3-butanedione), a result supporting the conclusion that the N-site is modified. Even if the modification is not necessarily restricted to a unique residue, the present data strongly suggest that glutathione reductase contains an essential arginine in the binding site for pyridine nucleotides (N-site).

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