Effect of Glutamate on the Formation of Mixed Disulfides by Plasma Proteins *in Vivo*[1] (34466)

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Cystine reacts with the free sulfhydryl groups of proteins to form a mixed disulfide (1–4) according to the following scheme:

$$\text{Prot–SH} + \text{Cys–S–S–Cys} \rightarrow \text{Prot–S–S–Cys} + \text{Cys–SH}.$$

The presence of such mixed disulfides has been reported in many tissues (1, 5, 6, 12).

The rate of this exchange reaction, when carried out either with native, denatured, or purified proteins, may be inhibited by glutamate and certain derivatives of this amino acid; no other amino acid[2] shows this inhibitory capacity (5). The inhibition is pH dependent and has an optimum at 7.2–7.6. The question then arose as to whether this inhibitory effect of glutamate on the formation of mixed disulfides could take place *in vivo* as well as *in vitro*.

Experimental Methods. The inhibition of the reaction of cystine with protein sulfhydryl groups by glutamate was tested by following the rate of addition of labeled cystine to the plasma proteins in adult female rats. Turnover of these labile half-cystine residues could be studied also at the same time. The binding of labeled cystine was measured in three groups of 5 rats each. The animals in Group 1 were given isotonic saline; those in Group 2 received 0.2 *M* aspartate; those in Group 3 were given 0.2 *M* glutamate subcutaneously. All solutions were adjusted to pH 7.4 prior to administration. Five-ml portions of these solutions were injected at 45, 30, and 15 min before the subcutaneous administration of 197 μCi of ^{35}S-L-cystine (sp act = 47 mCi/mmole) and again 15 min later. One-ml blood samples were drawn from the tail vein at 30-min intervals up to 2 hr following the administration of the labeled cystine. After centrifugation, the plasma was lyophilized and stored at 4° until analytical procedures could be carried out.

The dried plasma samples were made up to an approximately 2% solution. The proteins in each sample then were subjected to successive fractional precipitations with 0.5, 0.75, 0.875, and 1.0 saturated Na_2SO_4 at room temperature. The precipitated proteins in each fraction were centrifuged down, dissolved in water, and reprecipitated with the same concentration of Na_2SO_4. The reprecipitated proteins finally were dissolved in 0.1 *M* $NaHCO_3$. One aliquot of the protein solutions was dialyzed against running water for 24 hr to remove compounds of low molecular weight, including the ^{35}S-cystine present as the free amino acid. The labeled cystine remaining in this dialysand is bound to the proteins either by peptide bonds or by disulfide bridges exclusively (disulfide-bound half-cystine) or both. A second aliquot of the solution of plasma protein fractions was dialyzed against 400 vol of 0.03 *M* sodium sulfite at pH 8–9. Sulfite reacts with both the free sulfhydryl groups and disulfide bridges to form thiosulfate derivatives (1, 7, 8). At the same time, the cysteine derivative, arising from the half-cystine residues attached to the protein by disulfide bonds only, dialyzes away leaving behind in the dialysand only the labeled cystine bound into the protein by peptide bonds. The amount of disulfide-bound half-cystine which had been associated with the plasma proteins is calculated from

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[2] Under certain experimental conditions, aspartate has about 5—10% the inhibitory capacity of glutamate.

the difference in activity between the dialysand obtained from dialysis against water and that against the sulfite solution.

After adjusting the pH of the dialysands to 8.6 with 0.5 *M* Na_2CO_3, aliquots of both dialysands from each salt-precipitated protein fraction were separated into the 4 principal plasma protein components (albumin; α-; β-; γ-globulins) by electrophoresis on a Beckman Microzone apparatus. The relative proportion of the different protein components in each fraction was measured on a Beckman densitometer and integrator after staining with ponceau S. The actual amount of each protein component in the various fractions was calculated from the total tyrosine content (9) in the fraction and the known tyrosine content of each type of plasma protein.

The ^{35}S activity in 1.0-ml aliquots of protein solution was measured with a Beckman LS-250 liquid scintillation spectrometer. The fluor contained 0.25 Cab-O-Sil and 0.04% PPO in dioxane. The ^{35}S activity in each protein component of the plasma proteins was calculated from measurement of ^{35}S activity in the protein fractions precipitated with different concentrations of Na_2SO_4, using the following 4 simultaneous equations:

$$p = a_1A + b_1B + c_1C + d_1D, \quad (1)$$

$$q = a_2A + b_2B + c_2C + d_2D, \quad (2)$$

$$r = a_3A + b_3B + c_3C + d_3D, \quad (3)$$

$$s = a_4A + b_4B + c_4C + d_4D, \quad (4)$$

where: p, q, r, s = ^{35}S activity measured in the total protein of the fractions precipitated by 0.5, 0.75, 0.875, and 1.0 saturated Na_2SO_4, respectively. a_1, a_2, a_3, a_4 = concentration of albumin in fractions precipitated by 0.5, 0.75, 0.875, and 1.0 saturated Na_2SO_4, respectively. b_1, b_2, b_3, b_4 = concentration of α-globulins in fractions precipitated by 0.5, 0.75, 0.875, and 1.0 saturated Na_2SO_4, respectively. c_1, c_2, c_3, c_4 = concentration of β-globulins in fractions precipitated by 0.5, 0.75, 0.875, and 1.0 saturated Na_2SO_4, respectively. d_1, d_2, d_3, d_4 = concentration of γ-globulins in fractions precipitated by 0.5, 0.75, 0.875, and 1.0 saturated Na_2SO_4, respectively. A, B, C, D = counts/min/mg of protein bound to albumin, α-globulins, β-globulins, and γ-globulins, respectively.

Results and Discussion. The above experiments yielded information about two aspects of the metabolism of plasma proteins. From the change in the amount of labeled cystine bound to protein by peptide bonds, the rate of formation of each component of the plasma proteins could be determined. The binding and disappearance of the ^{35}S half-cystine bound to the proteins through disulfide bridges only is a measure of the formation and turnover of mixed disulfides.

The rate of synthesis of each of the components of the plasma proteins was not altered significantly by the administration of either aspartate or glutamate as compared to the rate observed in rats receiving saline only. We show only the data for bovine albumin (Fig. 1) since the data for each of the globulins tell essentially the same story.

Although glutamate had no effect on the rate of synthesis of plasma proteins, it drastically affected the rate of formation of mixed disulfides with these proteins. Figure 2 shows the rate of binding and removal of labeled half-cystine in the α-globulin fraction of plasma proteins. The administration of aspartate does not change the rate of formation of

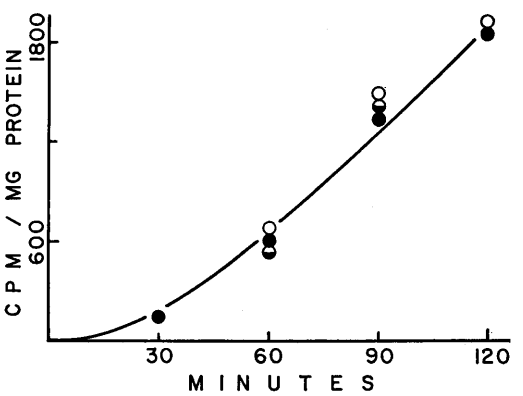

FIG. 1. The rate of formation of serum albumin in rats measured as counts/min/mg of protein against time after administration of 197 μCi of ^{35}S-L-cystine. The activity shown in this plot represents only the half-cystine bound into the protein by peptide bonds. (○), rats given 0.16 *M* saline; (◒), 0.2 *M* aspartate; (●), 0.2 *M* glutamate.

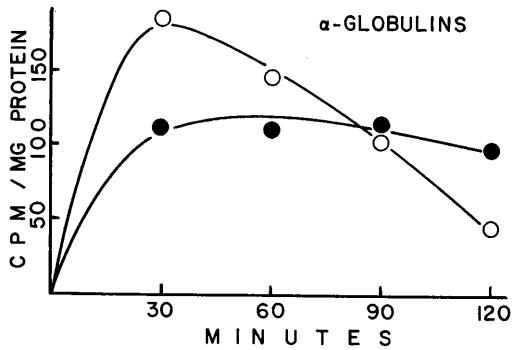

FIG. 2. The rate of binding and turnover of labeled cystine to α-globulins in the plasma of rats shown in terms of counts/min/mg of protein against time after the administration of 197 μCi of ^{35}S-L-cystine. The activity in this plot represents labeled cystine bound to the protein by disulfide bridges only. (○), the data derived from the α-globulins in rats receiving 0.16 *M* saline; essentially identical data were obtained from rats given 0.2 *M* aspartate; (●), data from rats given 0.2 *M* glutamate.

mixed disulfides from that seen in the control animals receiving saline. However, as shown glutamate significantly reduces the rate of mixed disulfide formation. The same picture was observed for the β-globulins (Fig. 3) and the γ-globulins (Fig. 4). At low concentrations of sulfhydryl groups it is difficult to observe the inhibitory effect of glutamate. Since serum albumin contains only one sulfhydryl group, it was not possible to find unequivocal inhibition by glutamate on the

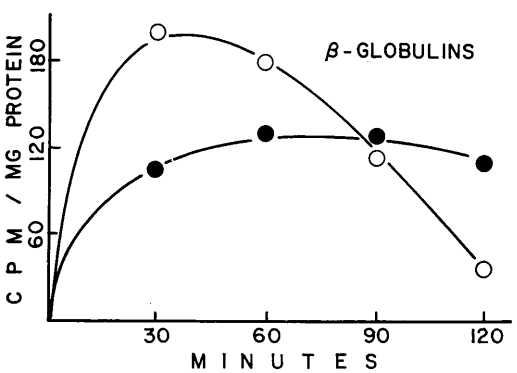

FIG. 3. The rate of binding and turnover of labeled cystine to β-globulins in rat plasma presented in terms of counts/min/mg of protein against time after administration of 197 μCi of ^{35}S-L-cystine. Symbols as in Fig. 2.

formation of mixed disulfides with this component of the plasma proteins.

The formation and turnover of mixed disulfides in proteins has been reported to be catalyzed by two different types of enzymes; one is a disulfide–sulfhydryl interchange enzyme (10); the other carries out an analogous reaction but is an oxido–reductase requiring NADPH (11). Neither of these enzymes has been demonstrated to be present in the plasma. It then seems likely that the formation of disulfide-bound half-cystine bridges by proteins in the plasma is largely, if not entirely, brought about by the same mechanism as that operating *in vitro*.

The fact that the labeled mixed disulfide bridges persist longer in the plasma proteins

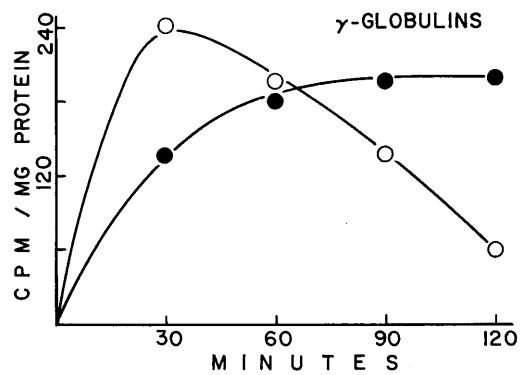

FIG. 4. The rate of binding and turnover of labeled cystine to γ-globulins in rat plasma in terms of counts/min/mg of protein against time after administration of 197 μCi^{35}S-L-cystine. Symbols as in Fig. 2.

of rats given glutamate than those receiving aspartate (Figs. 2, 3, and 4) indicates that glutamate inhibits not only the reaction of labeled cystine with the sulfhydryl groups of proteins but also the reverse reaction by which the ^{35}S is removed from the protein. Since inhibition of the forward reaction, shown above, is the same *in vivo* and *in vitro* (5), it seems likely that the reverse reaction will also be found to be inhibited by glutamate *in vitro*.

Summary. The administration of glutamate to rats decreases the rate of reaction between labeled cystine and the sulfhydryl groups of plasma globulins to form mixed disulfides. The rupture of these mixed disul-

fides bridges also appears to be inhibited by glutamate.

1. Williamson, M. B. and Clark, G. H., Arch. Biochem. Biophys. **144**, 314 (1966); Life Sci. **5**, 613 (1966).

2. Pihl, A., Eldjarn, L., and Nakken, K. F., Acta Chem. Scan. **12**, 1357 (1958).

3. Pihl, A., Lange, R., and Anders, E., Acta Chem. Scand. **15**, 1271 (1961).

4. Jakovlev, V. G. and Isupova, L. S., Radiobiologica **4**, 244 (1964).

5. Williamson, M. B. and Clark, G. H., Proc. Soc. Exptl. Biol. Med. **131**, 820 (1969).

6. Williamson, M. B., Clark, G. H., and Sorensen, M., Arch. Biochim. Cosmetol. **11**, 9 (1968).

7. Davis, R. E., Louis, J. B., and Cohen, A., J. Chem. Phys. **88**, 1 (1966).

8. Cecil, R. and McPhee, J. R., Biochem. J. **60**, 496 (1955).

9. Folin, O. and Ciocalteu, V., J. Biol. Chem. **73**, 627 (1927).

10. Fuchs, S., DeLorenzo, F., and Anfinsen, C. B., J. Biol. Chem. **242**, 398 (1967).

11. Spolter, P. D. and Vogel, J. M., Biochim. Biophys. Acta **167**, 525 (1968).

12. DeLorenzo, F. and Molea, G., Biochim. Biophys. Acta **146**, 593 (1967).

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