CATALYSIS OF FORMATION OF MIXED DISULFIDES BETWEEN CYSTINE AND β-GLOBULINS BY COPPER IONS*

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Summary

The ability of glutamate to inhibit the reaction between cystine and proteins to form mixed disulfides appears to be due to the formation of a complex between the copper ions and the amino acid. The copper ion seems to be the only divalent ion which can accelerate the rate of formation of the mixed disulfide.

The formation of mixed disulfides by an exchange reaction between proteins and either thiols or disulfides has been shown to take place *in vitro* (1-5); evidence is available to indicate that the same reactions may take place *in vivo* also (6-8). Although several enzymes have been identified which catalyze these, or analogous reactions(9-11), they may take place, even *in vivo*, without enzymatic intervention (7).

The binding of cystine to native and denatured tissue proteins, as well as to purified proteins, to form the mixed disulfide is inhibited by low concentrations of glutamate (2). Both the binding of ^{35}S-cystine to plasma proteins *in vivo* and the turnover of the half-cystine in the mixed disulfide appear to be inhibited by glutamate (7).

Plotting the reciprocal of inhibition against the recip-

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rocal of glutamate concentration at different pH levels gives a pattern which is typical for non-competitive inhibitors (2). This prompted us to suspect that glutamate is only indirectly involved in the inhibition of mixed disulfide formation. Glutamate is essentially the only amino acid which displays this inhibitory capacity*. This, added to the fact that glutamate derivatives in which either or both carboxyl groups are blocked do not act as inhibitors, while N-derivatives are as potent as the free amino acid, suggested that the complexing of divalent ions might be implicated in the inhibitory activity.

Experiments were undertaken to test whether the effect of glutamate could be attributed to the binding of various divalent ions which might be required for the exchange reaction. To 3.0 ml of solutions of bovine β-globulins (Fraction III) in 0.1 M tricine buffer at pH 7.4 was added 1.0 ml of 0.003 M $CuSO_4$ or other divalent salts in buffer, adjusted to pH 7.35-7.4. Another 1.0 ml of 0.045 M glutamate (or EDTA) in the same buffer was added. After the addition of 1.0 ml of buffer containing 0.001 μCi of ^{35}S-L-cystine (sp. act. = 18.5 mCi/mM), the reaction mixtures were incubated at room temperature for 120 minutes. The solutions were then transferred to narrow bore tubing and dialyzed against 4 liters of water containing 10 ml of 0.1 M EDTA and 10 ml of 0.5 M $NaHCO_3$ for 20-22 hours at 4° C with vigorous shaking. The ^{35}S activity in aliquots of the dialysand were measured with a gas-flow end-window G-M tube. The protein concentration was determined by the method of Lowry (12).

The results of several experiments are summarized in

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* Under certain circumstances, aspartate exhibits about 5-10% of the inhibitory capacity of glutamate.

TABLE I

EFFECT OF DIVALENT IONS ON BINDING OF CYSTINE TO PLASMA β-GLOBULINS

Reaction Mixture	Counts/Min Mg Protein
Control	1855 ± 50
Control + Glutamate (0.0075 M)	970 ± 45
Control + E D T A (0.0075 M)	375 ± 60
Control + $CuSO_4$ (0.0005 M)	2465 ± 75
Control + $CuSO_4$ + Glutamate	1180 ± 45
Control + $CuSO_4$ + E D T A	425 ± 45
Control + $ZnCl_2$ (0.005 M)	1850 ± 70
Control + $ZnCl_2$ + Glutamate	1020 ± 55
Control + $ZnCl_2$ + $CuSO_4$	2560 ± 85
Control + $CaCl_2$ (0.005 M)	1780 ± 65
Control + $MgSO_4$ (0.005 M)	1840 ± 70
Control + $CoSO_4$ (0.005 M)	1810 ± 55
Control + $MnCl_2$ (0.005 M)	1080 ± 60
Control + $FeSO_4$ (0.005 M)	965 ± 40
Control + $HgCl_2$ (0.005 M)	850 ± 55

The control reaction mixture contained 2.5-2.9 mg/ml of β-globulins (Fraction III) and 0.00016 μCi/ml of labeled cystine.

Table I. Not only glutamate but also EDTA significantly inhibit the binding of ^{35}S-cystine to the protein to form the mixed disulfide. The addition of copper ions results in a marked increase in the rate of formation. The inhibitory effect of glutamate, even when copper ions are added to the reaction mixture, is a strong indication that this ion acts

as a catalyst for the exchange reaction. It seems probable that the effect of glutamate reported earlier (2,7) was due to the presence of Cu^{++} ions as an impurity in the protein preparations used in previous experiments.

None of the other ions tested, even at 10 times the concentration of Cu^{++}, had any effect on increasing the rate of mixed disulfide formation. In fact, the exchange reaction is inhibited by manganous, mercuric and ferrous ions; the latter ion is inhibitory whether the reaction mixture is exposed to air or tested under nitrogen. Undoubtedly, the mechanism for this reaction is different from that due to glutamate or EDTA. It seems probable that the inhibitory effect of these ions is related to their binding to the sulfhydryl groups in the protein so that cystine cannot enter into the exchange reaction with them.

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