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Silver Complex Formation Equilibria of Corticotropin Fragments

B. NOSZÁL and K. BURGER

Department of Inorganic and Analytical Chemistry of the L. Eötvös University, P.O. Box 123, H-1443 Budapest, Hungary

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The fourth N-terminal amino acid in the peptide chain of corticotropin is methionine [1, 2]. The oxydation of its thioether sulphur atom to sulphoxide results in a dramatic decrease in the biological activity of the molecule [3]. The aim of our investigation was to elucidate the electron pair donor ability of this thioether sulphur in the macromolecule on the basis of its silver ion coordination.

The silver complex formation of three Corticotropin [ACTH] fragments – the N-terminal 32, 28 and 4 amino acid containing $ACTH_{1-32}$, $ACTH_{1-28}$ and $ACTH_{1-4}$ – was studied in aqueous solutions of pH 1.0, 2.0, 3.0 and 5.0, respectively. Since some donor atoms of ACTH are protonated, others are non-protonated in the pH-range of this study, the silver complex formation is composed of pH-independent and pH-dependent steps. To separate these processes not only the silver ion activity but also the pH of the solution was measured, and the pH-change due to silver coordination was compensated by using a sodium hydroxide standard solution.

A three-electrode potentiometric method [4] with a silver, a glass and a silver–silver chloride reference electrode (the latter placed in a Wilhelm bridge [5]) served this purpose.

The equilibrium constants were calculated using a computer program. The analytical concentrations (total peptide and silver concentration), silver ion activity, pH and the sodium hydroxide amount needed to keep the pH constant, were the primary experimental data.

The equilibrium measurements have shown that, on increasing the silver ion concentration in solutions containing the peptide, the coordination of the first silver ion is well separated from the successive binding of the next silver ions. This primary process was thoroughly investigated.

The corresponding equilibrium data are presented in Table I. The striking similarity of the stability constants for the three different corticotropin fragments indicate that the silver ion must be bound by identical functional groups in the different ACTH fragments. Since the N-terminal four amino acid residues containing $ACTH_{1-4}$ were the smallest among the peptides investigated, the donor groups situated in this part of the macromolecules must be considered as taking part in this coordination process. These are: the terminal amino and the tyrosine (2) phenolic hydroxy group and the methionine (4) tioether sulphur donor atom.

The pH dependence of the coordination of the first silver ion (measured by the silver electrode in solutions of different, but constant pH) and the amount of protons released in the course of complex formation indicated the overlapping of two equilibria: (a) the pH independent coordination of silver ion presumably to the methionine (4) sulphur; (b) a pH dependent process, most probably the coordination of the terminal amino group by the silver bound in the previous step to the methionine residue of the peptide (the closing of a chelate ring of rather unusually great size favoured by the linear *sp* hybridization of the coordinated silver).

In solutions of pH $\leqslant 3$, only process (a) described by equation

$$Ag^+ + HP \rightleftharpoons AgHP^+ \qquad (a)$$

and characterized by the value of K_S in the Table takes place. A further increase of the pH results in the appearance of the pH-dependent process (b):

$$AgHP^+ \rightleftharpoons AgP + H^+ \qquad (b)$$

The bonding strength of silver in AgP is characterized by

$$K_N = \frac{[AgP]}{[Ag^+]\,[P^-]}$$ in the Table.

The concentration $[P^-]$ was calculated from the total peptide concentration using the protonation constants [6] of the terminal amino groups of the

TABLE I. Stability Constants of the Silver Complexes of ACTH Fragments.

Peptide	lg K_S			lg K_N
pH	1	2	3	5
$ACTH_{1-4}$	3.50 ± 0.04	3.64 ± 0.04	3.56 ± 0.05	5.10 ± 0.1
$ACTH_{1-28}$	3.65 ± 0.1		3.63 ± 0.05	5.50 ± 0.1
$ACTH_{1-32}$	3.72 ± 0.1		3.58 ± 0.04	5.22 ± 0.1

corresponding peptides. The concentration- and pH-independence of the K_N values strongly support the correctness of the assignment of the terminal amino groups in process (b).

To confirm the assignment of the tioether sulphur atom in both processes (a) and (b), in one of the peptides ($ACTH_{1-28}$) this sulphur was oxidised to sulphoxy group preventing in this way its coordination to silver. The silver complex formation study of this oxidised peptide reflected a dramatic decrease in the stability of the silver complex, thus proving the correctness of the assignment.

The great difference in the values of the stability constants K_N and K_S ($K_N > K_S$) supports the assumption that the peptide acts in process (a) as a monofunctional ligand, and the chelate effect causes the increased stability of the species formed in process (b).

According to the data in the Table both constants are independent of the size of the peptide indicating that the parts of the molecule which do not interact directly with the coordinated silver have no effect on the stability of these complexes.

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