of 1-phenoxy-octa-2E,7-diene — a readily accessible co-oligomer of butadiene and phenol.

LITERATURE CITED

1. M. Jacobson, K. Ohinata, D. L. Chambers, W. A. Jones, and M. S. Fujimoto, J. Med. Chem., 16, 248 (1973).
2. G. Jones, M. A. Acquadro, and M. A. Carmody, J. Chem. Soc. Chem. Commun., 206 (1975).
3. O. P. Vig, M. L. Sharma, M. Gakhar, and N. Malik, Indian J. Chem., B19, 356 (1980).
4. E. J. Smutny, J. Am. Chem. Soc., 89, 6793 (1967).
5. L. I. Zakharkin, and T. B. Agakhanova, Izv. Akad. Nauk SSSR, Ser. Khim., 1400 (1979).
6. H. C. Brown, E. F. Knights, and G. C. Scouten, J. Am. Chem. Soc., 96, 7765 (1974).
7. H. C. Brown, K. J. Murray, L. J. Murray, J. A. Snover, and G. Zweifel, J. Am. Chem. Soc., 82, 4233 (1960).
8. M. Jacobson, I. Keiser, D. L. Chambers, D. H. Miyashita, and C. Harding, J. Med. Chem., 14, 236 (1971).
9. P. Saglio, E. Priesner, C. Descoins, and M. Gallois, C. R. Acad. Sci., Paris, D284, 2007 (1977).
10. D. L. Struble and C. E. Lilly, Can. Entomol., 109, 261 (1977).

IDENTIFICATION OF CATIONS IN THE ACTIVE CENTERS OF THE CARBOXYPEPTIDASE OF *Streptomyces griseus* AND THE AMINOPEPTIDASE OF *Aspergillus oryzae*

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It has been shown by atomic adsorption spectroscopy that the active center of the molecule of the carboxypeptidase of *Streptomyces griseus* contains 1 zinc atom and that of the aminopeptidase of *Aspergillus oryzae* 1 cobalt atom. The latter was also confirmed by the ESR method. In addition to the cobalt, 0.3 g-atom of strongly bound zinc, upon the pressure of which the enzymatic activity does not depend, has been found in the aminopeptidase.

As a rule, the activity of peptidases depends on the presence of bound metals — magnesium, cobalt, calcium, zinc, manganese, etc. Their roles in enzymatic reactions of peptidases are extremely diverse [1–3]. The metal present in the active center performs the function of a "bridge" binding the substrate with the enzyme in the formation of the intermediate complex, acts on the substrate, and creates or stabilizes a definite conformation of the molecule that is necessary for catalysis.

We give the results of a determination of the amounts of metal ions in the active centers of the carboxypeptidase of *Streptomyces griseus* and the aminopeptidase of *Aspergillus oryzae*.

The enzyme preparations were additionally purified by dialysis against double-distilled water at +4°C. To obtain the apoenzymes, o-phenanthroline was added to the enzyme solution before dialysis. After 3 h, the enzyme had lost its activity completely, while in a control the activity was almost completely retained. The excess of complex-forming agent and the metal cations bound to it were eliminated from the solution of the apoenzyme by dialysis. The apoenzyme obtained was analyzed in a similar manner to the initial preparation. In a native preparation of the carboxypeptidase of *Streptomyces griseus*, 1.15 atoms of zinc

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TABLE 1. Amounts of Metals in Peptidase Molecules

Enzyme	Method of determination					Mol. wt. of enzyme, amu
	atomic absorption spectroscopy			ESR spectroscopy		
	Zn	Co	Mn	Co	Mn	
	g-atom/mole of protein			g-atom/mole of protein		
Aminopeptidase of Aspergillus oryzae	0,3	1,1	Tr.	0,9	Tr.	60000
Carboxypeptidase of Streptomyces griseus	1,15	Tr.	Tr.	Tr.	Tr.	29000
Carboxypeptidase A.	1,05	Tr.	Tr.	—	—	34600

TABLE 2. Parameters of the Determination of the Elements in Peptidases by Atomic Absorption

Element	Source of monochromatic radiation	Wavelength, nm	Optical slit, nm	Parameter					
				drying		ashing		atomization	
				t″	t, C	t″	t, C	t″	t, C
Zinc	Hollow-cathode lamp for Zn	213.9	0.7	30	100	20	300	15	1900
Cobalt	Hollow-cathode lamp for Co	240,7	0 2	30	100	40	1100	20	2600
Manganese	Hollow-cathode lamp for Mn,	279,5	0.2	25	100	30	1000	15	2600

calculated to a molecule of the protein, taking the molcular weight as 29,000 [4], was found by atomic absorption. Only traces of it were detected in the apoenzyme. Of other metals, traces of cobalt and manganese were found both in the native enzyme and in the apoenzyme.

Traces of cobalt and manganese were found in the same preparations by ESR spectroscopy. No copper or iron was detected either at room temperature or at −273°C (Table 1).

Thus, the active center of the carboxypeptidase of *Str. griseus* contains one zinc atom.

The aminopeptidase of *Asp. oryzae* was investigated in a similar manner. Cobalt and zinc were detected in the initial preparation by atomic absorption spectroscopy (see Table 1). The amount of cobalt was 1.1 g-atom and that of zinc 0.3 g-atom per one mole of protein if its molecular weight is taken as 60,000 [5].

Using ESR spectroscopy at −273°C, 0.9 g-atom of cobalt per mole of protein was found in the native aminopeptidase. No copper, iron, or manganese cations were detected.

In the apoenzyme that had lost its activity completely, the concentration of Co^{2+} had been halved while that of Zn^{2+} had scarcely changed. The cobalt is apparently located in the active center of the aminopeptidase and the zinc is strongly bound to other sections of the molecule.

As we have shown previously, at molar concentrations of cations exceeding the concentration of enzyme by factors of 10–10^3 the activity of the aminopeptidase is 84% restored by Mn^{2+}, 92% by Zn^{2+}, and 270% by Co^{2+} (taking the activity of the native preparation as 100%):

Cations	Concentration of the salt, M	Concentration of the protein, M	Activity %
Co^{2+}	10^{-4}	10^{-6} —10^{-7}	270
Zn^{2+}	10^{-4}	10^{-6} —10^{-7}	92
Mn^{2+}	10^{-4}	10^{-6} —10^{-7}	84

However, in this case the metal may not be included only in the active center. The presence of several centers of binding of metals has also been discussed for the thermophilic aminopeptidase of *Bac. stearothermophilus* and the leucine aminopeptidase of the crystalline lens of the bovine eye [3]. It is possible that in this case the zinc ions are bound with the sulfhydryl groups, as has been shown for the case of carboxypeptidase A and leucine aminopeptidase [6, 7].

EXPERIMENTAL

Trace elements were determined by atomic absorption spectroscopy on a Perkin-Elmer model 403 instrument with a HGA-74 carbon atomizer. The sensitivity of the method was 0.02 μg/ml. The absorption was recorded for the absorption lines of Zn^{2+} at 213.9 nm, Mn^{2+} at 279.5 nm, and Co^{2+} at 240.7 nm. Cathode lamps were used as sources of monochromatic radiation. The spectral slit width of the monochromator for Mn^{2+} and Co^{2+} was 0.2 nm and for Zn^{2+} 0.7 nm (Table 2).

Freshly-prepared dilute solutions of pure salts of the corresponding metals were used as standards.

Solutions of the initial homogeneous preparations, the preparation of which has been described previously [4, 5] were dialyzed against double-distilled water at +4°C in quartz capillaries with frequent changes of the water for 48 h. The carboxypeptidase activities in the enzyme solutions obtained were determined from the hydrolysis of benzyloxycarbonylglycylleucine, and aminopeptidase activities from the hydrolysis of leucylglycylglycine [8].

The apoenzymes were obtained by treating the proteins with o-phenanthroline, for which purposes 30 mg of o-phenanthroline was added to 30 mg of the initial protein dissolved in 3 ml of double-distilled water and the mixture was incubated at room temperature for 24 h. Subsequent dialysis for 48 h was carried out in order to eliminate the excess of complex-forming agent and its complex completely.

The amounts of Co^{2+}, Zn^{2+}, and Mn^{2+} as the most probable metals for the active centers of peptide hydrolases were determined in the initial proteins and the apoenzymes. The ESR spectra of aqueous solutions of the enzymes were recorded in an instrument of the TSN-254 type in the 3-cm range at room temperature in a plane cell, and those of frozen solutions at −273°C (77°K) in 3-mm sealed quartz capillaries.

SUMMARY

It has been shown by atomic-absorption and ESR-spectroscopic methods that the active center of the molecule of the carboxypeptidase of *Streptomyces griseus* contains one atom of zinc, and the active center of the aminopeptidase of *Aspergillus oryzae* has been found to contain one atom of cobalt.

LITERATURE CITED

1. S. R. Himmelhod, in: Methods in Enzymology, Vol. 19, G. E. Perlman and L. Lorand, eds., Academic Press, New York (1970), p. 598.
2. R. J. Delange and E. L. Smith, in: The Enzymes, P. D. Boyer, ed., Academic Press, New York, Vol. III (1971), p. 81.
3. J. Lasch, Molekulare Grundlagender Wirkungsweise von Metallopeptiden, Wiss. Z. Martin-Luther-Univ., Halle-Wittenberg, Math.-Naturwiss. Reihe, 27, No. 2, 101 (1978).
4. A. S. Pilyavskaya, "The isolation and properties of the carboxypeptidase of *Streptomyces griseus*," Author's abstract of Candidate's dissertation, Kiev (1977), p. 23.
5. M. V. Kolodzeiskaya, S. V. Verbilenko, and L. A. Konoplich, Ukr. Biokhim. Zh., 51, No. 1, 56 (1979).
6. J. E. Coleman and B. B. Vallee, J. Biol. Chem., 236, No. 8, 2244 (1961).
7. T. L. Coombs, Y. P. Felber, and B. B. Vallee, Biochemistry, 1, No. 5, 899 (1962).
8. M. V. Kolodzeiskaya, S. V. Verbidenko, and L. A. Konoplich, Usp. Biokhim. Zh., 52, No. 1, 92 (1980).