Vol. 96, No. 3, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 16, 1980 Pages 1384-1392

SPECTROSCOPIC INVESTIGATION OF FeMo-COFACTOR. COENZYME A AS ONE OF THE PROBABLE COMPONENTS OF AN ACTIVE SITE OF NITROGENASE

L.A.Levchenko, O.S.Roschupkina, A.P.Sadkov, S.A.Marakushev, G.M.Mikhailov, Yu.G.Borod'ko

Institute of Chemical Physics, USSR Academy of Sciences, 142 432 Chernogolovka, Moscow Region, U.S.S.R.

Received September 2,1980

SUMMARY

FeMo-cofactor, isolated from nitrogenase of *Azotobacter vinelandii*, has been studied by IR-, Raman-, UV-, and AUGER-specproscopies. CoenzymeA has been supposed to be present as a component of the FeMo-cofactor. The pattern, explaining the possible functional role of the coenzymeA - lipoic acid couple in the dinitrogen reduction by nitrogenase, has been put forward.

INTRODUCTION

A whole number of investigations has been devoted to the structural and functional role of MoFe-protein of nitrogenase as it might throw some light on the mechanism of biological fixation of nitrogen. The isolation of low-weight FeMo-cofactor (FeMo-co) (1), containing molybdenum, iron, and acid-labile sulfur in the ratios 1:(6-8):1, simplifies substantially the problem of compositional and structural analyses of FeMo-cluster (FeMoS) of nitrogenase active site (2-7). According to papers (7,8) FeMo-co contains neither peptide basis nor individual amino acids. However, it was found that organic unidentified compound with NH_2-group was a component of FeMo-co (8).

Recently in (9) there has been an S-containing organic component found, which is coordinated with Fe, and a supposition

0006-291X/80/191384-09$01.00/0

has been made, that it might be lipoic acid, probably in the complex with coenzymeA (CoA). Indirectly on the ground of thin-layer chromatography experiments lipoic acid only has been detected (9), but the other organic compounds were not identified.

In the present work we have made an attempt to clarify, whether CoA or any adenosine-containing components are present in FeMo-co, using IR-, Raman-, UV-, and AUGER-spectroscopies.

MATERIALS AND METHODS

FeMo-co was isolated from crystalline MoFe-protein as in (1). Its catalytic activity was determined by C_2H_2 reduction in the presence of $NaBH_4$ and after subsequent recombination with defect nitrogenase[4]of mutant of *Azotobacter vinelandii* UW-45 in the presence of ATP-generating system (1,10). The Fe- and Mo-contents were defined by atomic absorption spectroscopy. The number of SH-groups was estimated by amperometric titration (11), labile sulfur was detected as in (12). The degree of FeMo-co homogeneity was evaluated by thin-layer chromatography on the SILUFOL plates. The cofactor dried till complete removal of the solvent anaerobically was used in our work (fraction I). To obtain some fractions of FeMo-co the dried powder (fraction I) was treated with chlorophorm:methanol (1:1) mixture for 30-60 min and then centrifugated for 30 min at about 3000g. Supernatant liquid was gethered and dried under anaerobic conditions (fraction II). The remaining residue was washed with chlorophorm-methanol mixture and dried again (fraction III).

Individual coenzymeA (Kyowa Hakko, Japan) and NAD, NADP (Reanal, Hungary) were investigated as references.

IR-absorpetion spectra were recorded on spectrophotometer PE-325, the samples were prepared as KBr-pellets. Raman spectra were excited with Ar^+-laser (5145 Å) and registered on spectrometer "Coderg PHO". UV-absorption spectra were recorded on spectrophotometer "Unicam SP 800", the samples were prepared as water or chlorophorm-methanol solutions, the thickness of an optical layer being 0.1 mm.

AUGER-spectra were recorded on spectrometer PHI-551 at vacuum 5×10^{-9} Torr in the test-chamber. The energy of electron was 3 kV and the current density was ca. 2×10^{-5} a cm^{-2}.

The line positions (kinetic energy of AUGER-electrons) were calibrated relative to KLL.C line, whose E_{kin} was defined as 272 eV.

The formamide solution of cofactor was put on the Al-foil, then dried at 150°C. The surface of the samples was subjected to special pretreatment by means of ion argon sputtering for a short period immediately before the measurements.

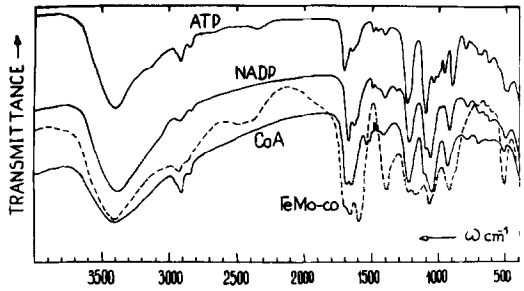

Figure 1: IR-spectra of FeMo-co, CoA, NADP and ATP (KBr-pellets)

RESULTS AND DISCUSSION

Chromatography of FeMo-co in N-methylformamide showed homogeneity of the investigated compounds. Catalytic activity of FeMo-co, measured with the addition of $NaBH_4$, was 30 nM C_2H_4 $min^{-1}nM^{-1}$ Mo and after the recombination with defect nitrogenase - 300 nM C_2H_4 $min^{-1}nM^{-1}$ Mo. The ratio Mo:Fe:S^{--}:SH was 1:8:6:8.

Vibration Spectra. Fig. 1 presents IR-spectra of FeMo-co (I), CoA∼S-Ac and NADP. The spectrum of the cofactor is more complicated because it contains greater number of bands. However it is noteworthy, that all intensive bands, characteristic of individual CoA or NADP, are in the FeMo-co spectrum, their coincidence being observed not only in their frequency, but also in intensity, i.e. it is very likely that FeMo-co contains a component with adenosine group.

Qualitative assignment of the most intensive bands in IR- and Raman-spectra of FeMo-co is given in Table I, where the vibrations of the following groups: Fe-S, C-S, P=O, COO^-, C=N, C=C, C=O, S-H, C-H, OH' and NH - are identified. It is noteworthy, that in IR-spectra of FeMo-co besides adenosine bands, there are intensive bands at 1600 and 1400 cm^{-1}, characteris-

Table I

Infrared and Raman Spectra of FeMo-co and CoA

FeMo-cofactor		CoenzymeA	NADP	ATP	
Raman (cm^{-1})	IR (cm^{-1})	IR (cm^{-1})	IR (cm^{-1})	IR (cm^{-1})	Assignment
	200				
227w*				235	
262m			318		δ(CCC)
368s	374				ν(Fe-S)
	395				
	415		405	420	
432w					
530w	524	516	516	516	
678s		720			ν(C-S)
	896			900	
	940	940	940	914	$\rho_r(CH_2)$
1045m	1050			968	
	1070	1076	1084	1100	
	1180	1120	1110	1120	
	1236	1234	1240	1254	ν(P=O)
	1394				$\nu_s(COO^-)$
	1604				$\nu_{as}(COO^-)$
	1670	1660	1640	1646	ν(C=N,C=C)
	1708	1692	1690	1712	ν(C=O)
	2450				ν(S-H)
	2850	2850	2850	2850	$\nu_s(CH_2,CH_3)$
	2920	2920	2940	2940	$\nu_{as}(CH_2,CH_3)$
	3400	3400	3400	3400	ν(OH,NH)

* w - weak, m - middle, s - strong

tic of stretching frequencies of carboxylate ion $\nu_{as}(COO^-)$, and $\nu_s(COO^-)$, respectively.

Electron spectra of FeMo-co(II) and CoA are given in Fig. 2. If the absorption band with λ_{max} = 262 nm is characteristic of CoA spectrum in water solution, then there is an absorption band with λ_{max} = 275 nm in FeMo-co(II) spectrum of chlorophorm-methanol solution. The shift into the long-wave region is caused either by the effect of dielectric properties of the solvent or by the coordination of the adenine ring to FeMoS.

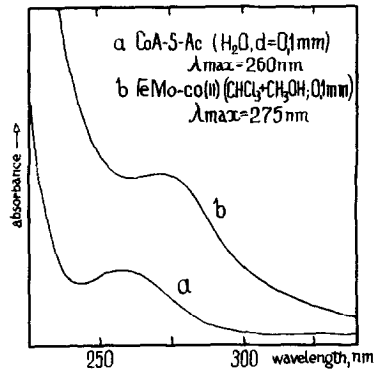

Figure 2: UV-spectra of FeMo-co and CoA (thickness 0.1 mm, CaF_2-cell)

AUGER-spectra allow to analyze the surface composition of the solid samples both qualitatively and quantitatively. Fig. 3 presents spectra of FeMo-co(I), (II), (III), CoA and lipoic acid. FeMo-co(I) spectrum contains such elements as N, C, S, O, P, and traces of Fe. The presence of Na-lines is artefact due to Na_2HPO_4 impurities, therefore the estimation of even rela-

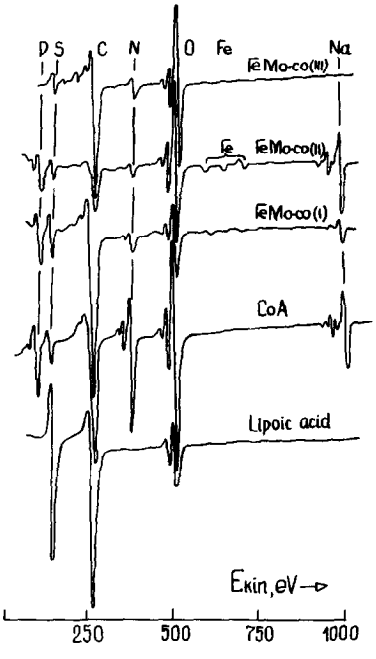

Figure 3: AUGER-spectra of FeMo-co fractions, CoA, and lipoic acid

tive concentrations of P, O, and S is difficult. The experimental data for N, C, S, and Fe are more informative. The estimation of relative concentrations of elements of FeMo-co, CoA, and lipoic acid are given in Table II, where concentration of nitrogen is taken for 7, which corresponds to its atomic concentration in individual CoA.

The sensitivity indexes for elements in AUGER-spectra are taken from (13).

It should be noted, that [Fe]/[S] ratio considerably changes in FeMo-co fractions. Thus [Fe]/[S] value in FeMo-co(I) is very small, whereas it is 10/7 in FeMo-co(II), that agrees well with the results of elemental analysis for iron, and acid labile sulfur $[Fe]/[S^{--}]$ = 8/6, obtained independently.

If the whole amount of FeMo-co nitrogen is supposed to belong to CoA, then the increase of the [S]/[N] ratio from 1/7 in CoA up to 7/7 in FeMo-co(I) evidences that at most one like CoA molecule is included in one FeMoS.

Table II

Relative Concentrations of Elements
Measured by AUGER-spectroscopy

Element / Compound		S	C	N	O	P	Na	Fe
FeMo-co(I)		7	130	7	20	14	14	traces
FeMo-co(II)		7	80	7	100	20	130	10
FeMo-co(III)		3	100	7	100	14	-	-
CoA∼S-Ac	exper.	1	16	6	9	3	7	-
	theor.	1	23	6	17	3	-	-
Lipoic Acid	exper.	2	7	-	1			
	theor.	2	8	-	2			

Thus the results of qualitative AUGER-analysis are not contraversary with the supposition that CoA is included into the FeMo-co composition, and the quantitative analysis shows that FeMo-co consists of organic compounds, besides CoA and FeMoS.

The functional role of coenzymeA in dinitrogen reduction is presented in Fig. 4. Within this model CoA~S-Ac probably takes part in acylation of lipoic acid, ligated to FeMoS and the energy stored up by macroergic thio-ether bond is further spent to increase the potential of the electron transferred from FeMoS to coordinated dinitrogen. The reformation of the initial state of CoA~S-Ac and Fe-S bonds takes place at the expense of ATP.

As it follows from (9), there are two FeMo-co units in a nitrogenase molecule. It is likely to assume that two Mo ions of adjacent FeMo-co are bridged with dinitrogen (14).Thus two chains of electron transfer operate and few electrons can jump simultaneously onto coordinated N_2 molecule. This multi-elect-

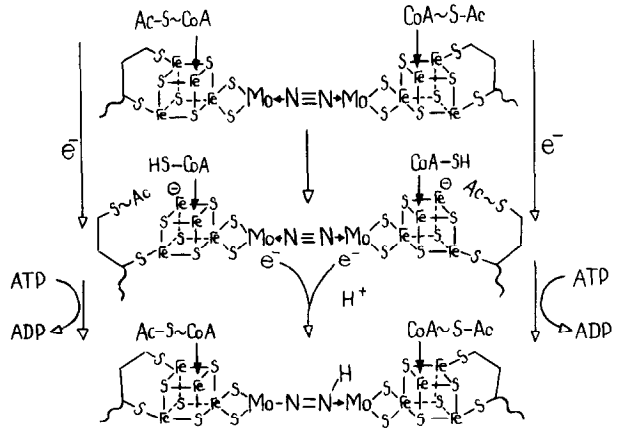

Figure 4: Pattern of probable role of CoA - lipoic acid couple in the dinitrogen reduction process in the active site of nitrogenase

ron reductive cycle is repeated again with subsequent formation of a hydrazine derivative and so forth.

The proposed scheme explains the CoA∼S-Ac role in the dinitrogen fixation process. The reactions of an acyl group transfer to lipoic acid are well known in biochemistry (15). However, the suggested scheme of interrelation of CoA∼S-Ac - lipoic acid couple with FeMoS and their role in dinitrogen reduction are, to our mind, new and unknown before. Therefore we intend to obtain further experimental confirmations.

We thank Dr. G.A.Kichigina for Raman measurements and Prof. A.E.Shilov for helpful discussion of the results.

REFERENCES

1. Shah, V.K., and Brill, W.S. (1977) Proc.Natl.Acad.Sci.USA, 74, 3249-3253
2. Cramer, S.P., Hodgson, K.O., Gillum, W.O., and Mortenson, L.E. (1978) J.Amer.Chem.Soc., 100, 3398-3402
3. Cramer, C.P., Gillum, W.O., Hodgson, K.O., Mortenson, L.E., Stiefel, E.J., Chisnell, J.R., Brill, W.J., and Shah,V.K., (1978) J.Amer.Chem.Soc., 100, 3814-3819
4. Rawlings, J., Shah, V.K., Chisnell, J.R., Brill, W.J., Zimmerman R., Münck, E., and Orme-Johnson, W.H. (1978) J.Biol. Chem., 253, 1001-1004
5. Huynh, B.H., Münck, E., and Orme-Johnson, W.H. (1979) Biochim.Biophys.Acta, 527, 192-203
6. Stephens, P.J., McKenna, C.E., Smith, B.E., Nguyen, H.T., McKenna, M.-C., Tomson, A.J., Delvin, F., and Jones, J.B. (1979) Proc.Natl.Acad.Sci.USA, 76, 2585-2589
7. Newton, W.E., Burgess, B.K., and Stiefel, E.J. (1980) Molybdenum Chemistry in Biological Significance, Eds. W.E. Newton and S.Otsuka, pp. 67 - 75 , Plenum Press, New York
8. Mitsova, I.Z., Skripkin, A.Yu., Gvozdev, R.I., Likhtenstein, G.I., Shishkov, A.V. (1980) Izv.Akad.Nauk SSSR, ser.biol., No. 6, 1193-1197
9. Levchenko, L.A., Sadkov, A.P., Marakushev, S.A., Raevskii, A.V., Pivovarova, T.S., Likhtenstein, G.I. (1980) Dokl. Akad.Nauk SSSR, in press
10. Shah, V.K., Chisnell, J.R., and Brill, W.J. (1978) Biochim. Biophys.Res.Communs, 81, 232-236

11. Kolthoff, J.M., and Harris, W.E. (1946) Ind.Eng.Chem., Analyt.Ed., 18, 161-166

12. Lovenberg, W., Buchanan, B.B., and Rabinowitz, T.S. (1963) J.Biol.Chem., 238, 3899-3913

13. Handbook of AUGER Electron Spectroscopy. Ed. Physical Electronics Industries,Inc.

14. Shilov, A.E. (1977) Biological Aspects of Inorganic Chemistry, Eds. A.W.Addison, W.R.Cullen, D.Dolphin, and B.R. James, pp. 197-236, "J.Wiley & Sons",

15. Torchinsky, Y.M. (1977) Sulfur in Proteins, pp. 211-213, Nauka, M.(USSR)