Reviews

The role of a P-cluster in the nitrogenase ATPase reaction

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Structural data on the nitrogenase complex of *Azotobacter vinelandii,* Av1 · $(Av2)_2$, stabilized by MgADP · AlF_4^- and, in particular, the structure and properties of a P-cluster involved in the nitrogenase ATPase reaction, were analyzed. The ATP-binding site and all nitrogenase metal clusters are arranged in one plane, the distances between the closest partners being 14—15 Å. The ATP-binding site in the Fe-protein, which decreases the half-reduction potential (E_m) of the [4Fe—4S]-cluster in Av2 to −0.43 V, does not affect the potentials of the P-cluster and Fe—Mo cofactor (FeMoco). Amino acids 74—95 in the β-subunit of Av1 "envelop" the P-cluster in Av1; therefore, the phosphate intermediate of the ATPase reaction of nitrogenase occurs apparently in the direct contact with the P-cluster. By increasing the acceptor properties of the P-cluster, this intermediate may favor the electron transfer from the Fe-protein to the P-cluster, thus bringing it into the super-reduced state.

Key words: nitrogenase, Fe-protein, Mo—Fe-protein, FeMoco, nitrogen fixation, energy coupling.

Nitrogenase (EC 1.18.6.1), an enzyme catalyzing the reduction of N_2 to NH_3, consists of two metalloproteins: a molybdenum-and-iron-containing protein (Mo—Fe-protein) and an iron-containing protein (Fe-protein), which functions as a MgATP-dependent electron donor with respect to the Mo—Fe-protein. The Mo—Fe-protein and Fe-protein from *Azotobacter vinelandii* are referred to as Av1 and Av2, respectively, while similar proteins from *Clostridium pasteurianum* are called Cp1 and Cp2, *etc*. The composition and structure of Av2 (see Ref. 1) and Av1 (see Refs 2—4) have been comprehensively characterized. The Av2 protein is a homodimer with a total molecular weight of ~60 kDa; it contains one [4Fe—4S] cluster incorporated symmetrically between two subunits.[1] The Av1 protein is a heterotetramer of two subunit types, $\alpha_2\beta_2$, with a total molecular weight of ~250 kDa; it contains two so-called P-clusters with the composition 8Fe—7S and two FeMoco-clusters with the composition Mo—7Fe—9S · homocitrate, which are substrate-binding and -reduction sites.[2,3]

A recent crystallographic analysis of Av1 with a 1.16 Å resolution showed the presence of an atom at the center of FeMoco, identified as nitrogen, coordinated to six iron atoms.[4] However, later it has been shown[5] that the central atom of FeMoco is carbon or oxygen rather than nitrogen. More comprehensive data about the redox poten-

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tials of the Av2 and Av1 [Fe—S]-clusters have been obtained.[6–10] Alternative electron donors for nitrogenase have been proposed, namely, the photodonors eosin—NADH[11] and 4′,5′-dibromofluorescein—NADH.[12] The kinetics of transfer of one and two electrons in nitrogenase with a photodonor has been studied.[13] The photodonors[11–15] and a natural electron donor flavodoxin[16] reduce Av2 in both the free state and a complex with Av1.

The electron transfer from Av2 to Av1 against the potential is the key step of the nitrogenase reaction. In the presence of dithionite, Av1 produces only one ESR signal, S = 3/2, typical of the one-electron reduction product of FeMoco ($FeMoco^N$). The half-reduction potential (E_m)* for the $FeMoco^N \rightleftarrows FeMoco_{ox.}$ transition is −0.042 V (see Ref. 7). In a process coupled with the ATP hydrolysis, Av2 transmits at least one electron to $FeMoco^N$ to give the so-called super-reduced FeMoco, $(*FeMoco)^{1-}$ (see Ref. 8); whereupon the signal S = 3/2 disappears from the ESR spectrum. According to experimental data, E_m for the $FeMoco^N \rightleftarrows (*FeMoco)^{1-}$ transition in Av1 varies from −0.6 to −1 V (see Ref. 17).

Unlike FeMoco, the P-cluster is diamagnetic in the presence of dithionite (the spin state S = 0). According to a publication,[9] this state corresponds to the ferroiron (+2) cluster, P^N, which can be reversibly oxidized to paramagnetic states, P^+, P^{2+}, and P^{3+}. The E_m values for the $P^N \rightleftarrows P^{2+}$, $P^N \rightleftarrows P^+$, $P^+ \rightleftarrows P^{2+}$, and $P^{2+} \rightleftarrows P^{3+}$ transitions at pH 8 are −0.307, −0.309, −0.309, and +0.09 V, respectively (see Ref. 9). The P^{2+} cluster exhibits an ESR signal typical of S = 3. Signals for the spin states S = 5/2 and S = 1/2 appear in the ESR spectrum of the P^+ cluster. Some data attest that the P-cluster is involved in the electron transfer within Av1. P-clusters are oxidized when nitrogenase reduces N_2 (see Ref. 18).

The authors of a publication[19] have concluded that the intramolecular electron transfer in nitrogenase is not an elementary reaction but includes, at least, two stages, namely, the electron transfer from Av2 to an unidentified site in Av1 (presumably, on the P-cluster) and subsequent reduction of FeMoco. Apparently,[20] during ATP hydrolysis the conformation of Av1 in the Av1·Av2 complex changes; this either facilitates the electron transfer from the P-cluster to FeMoco, after which the P-cluster accepts an electron from Av2, or makes the P-cluster an electron acceptor until the electron is transferred to FeMoco. It is known that MgATP induces conformational changes upon binding with Av2 (see Ref. 6). Upon the reactions with chelating agents, the reactivity of [4Fe—4S]-cluster increases. The ESR spectrum of the reduced [4Fe—4S]-cluster in Av2 changes: the shape of the ESR signal (S = 1/2) changes from rhombic to axial. The half-reduction potential E_m becomes more negative (by 0.12 V). The ^1H NMR spectrum of the reduced Av2 changes. The circular dichroism spectra also change.

If the P-cluster transfers the electron further to FeMoco during ATP hydrolysis, its redox potential should be sufficient for the transition of FeMoco into the super-reduced state, *i.e.*, not less than −0.6 V. However, it remains obscure how a cluster consisting of ferroiron could accept an additional electron, which would transform it into the super-reduced state with respect to P^N.

For the P-cluster, properties have been found that may be essential for its functioning.[3] Substantial differences between the structures of P^N and $P_{ox.}$ were revealed. In each of these states, the P-cluster is an [8Fe—7S]-cluster. In the oxidized state, it can be represented as a single cluster consisting of two S(1)-bridged clusters, *viz.*, [4Fe—4S] and [4Fe—3S]. The P-cluster undergoes a redox-dependent structural rearrangement, which can be coupled with electron or proton transfer to FeMoco. Interconversion between the oxidation states of the P-cluster was found; this included migration of two Fe atoms with replacement of their coordination to the protein by coordination to the central sulfur atom S(1). The oxidation of the P-cluster is accompanied by coordination of Ser β188 and the amide nitrogen of Cys α88 to the Fe atoms of the P-cluster. Since both ligands are protonated in the free state and can be deprotonated in the bound state, it has been suggested[3] that two-electron oxidation of the P-cluster may liberate two protons and that the change in the ligands in the P-cluster can result in coupled electron and proton transfer from the P-cluster to FeMoco. This hypothesis was supported in a publication[10] in which the electron transfer during P^{1+} oxidation to P^{2+} was shown to be accompanied by coupled proton transfer at physiological pH values.

A helical amino acid sequence binding the P-cluster ligand Cys α62 with Val α70 in the FeMoco region has been identified.[21] Presumably, this helix is a potential route for electron transfer directly from the P-cluster on FeMoco. The reduction center of substrates with FeMoco is a new-type cluster containing both Mo and Fe. In this cluster, each Fe—S—Fe group binds as bridges two subclusters, [4Fe—3S] and [1Mo—3Fe—3S], and forms two [4Fe—4S] faces. Val α70 is located at a short distance from one of these faces.[21]

Structural details of substrate binding to FeMoco and the sequence of electron and proton transfer to the substrate bound in the active site are still obscure. It is known that the general stoichiometry of the nitrogenase-catalyzed reduction of nitrogen can be described as follows:[22]

$$N_2 + 8\,H^+ + 8e^- + 16\,MgATP \rightarrow \rightarrow 2\,NH_3 + H_2 + 16\,MgADP + 16\,P_i{**}.$$

* E_m is the half-reduction potential at pH 7.0 *vs.* hydrogen electrode.

** P_i is inorganic phosphate.

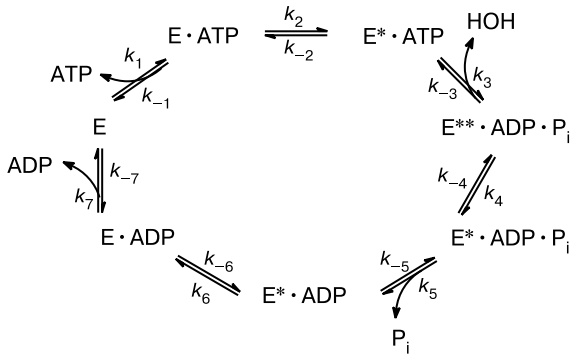

Fig. 1. Sequence of stages of ATP hydrolysis with myosin.[25] E is the myosin ATPase. The conformationally modified states of the enzyme are asterisked. Equilibrium constants: $K_1 = 4.5 \cdot 10^3$ L mol^{-1}, $K_2 \geq 2 \cdot 10^4$, $K_3 = 9.0$, $K_5 = 7.3 \cdot 10^{-3}$ mol L^{-1}, $K_6 = 3.5 \cdot 10^{-3}$, $K_7 = 2.7 \cdot 10^{-4}$, $K_{tot} = 7.5 \cdot 10^5$ mol L^{-1}. Rate constants for direct reactions (s^{-1}): $k_2 = 400$, $k_3 = 160$, $k_4 = 0.06$ (in the presence of actin 20), $k_6 = 1.4$. For the sake of simplicity, MgATP and MgADP were replaced by ATP and ADP.

The transport of each electron is associated with hydrolysis of at least two ATP molecules, which are bound to the Fe-protein as a Mg^{2+} complex.

Data have been reported indicating that the kinetic sequence of stages of MgATP hydrolysis with nitrogenase is the same as in myosin[25] (Fig. 1).[23,24] Stages 1 and 2 include energy accumulation as a strained conformation of the protein. The most pronounced changes in the standard free energies ΔF° occur upon the addition of ATP to ATPase, and the least pronounced changes take place upon the reversible —O—P— bond cleavage (see Fig. 1, stage 3). The elimination of products (ADP, P_i) requires energy expenditure.[26]

The reaction releasing the ATP hydrolysis products into the medium is the rate-determining stage; as in the case of other ATPases, this may be two-stage elimination of MgADP (stages 6 and 7 in Fig. 1). The change in the enzyme conformation with MgADP to the conformation corresponding to the initial enzyme is slow (stage 6) and is followed by fast removal of MgADP to the medium (stage 7). The reversion of the conformational changes that accompany ATP binding and cleavage underlies the energy transformation.[27] This takes place in the so-called "coupling" stage 4 (see Fig. 1). In the energy transformation systems in the complex with MgATP and MgADP, the active site is shielded from the medium, and in stage 4, ATP hydrolysis occurring in several steps is coupled with the reactions (not shown in Fig. 1) to which coupling is directed: in nitrogenase, this is electron transfer.

MgADP should remain bonded to the enzyme for a period of time required for the formation of $(*FeMoco)^{1-}$. Indeed, it has been reported[28] that the rate of MgADP removal from the nitrogenase molecule is low even in the first electron transfer step: the first MgADP molecule is detached from nitrogenase with $k_{eff} \leq 0.2$ s^{-1}, while the second one, with $k_{eff} \geq 0.6$ s^{-1}. After $(*FeMoco)^{1-}$ has formed, the nitrogenase conformation should return to the initial state. In nitrogenase, the formation of $(*FeMoco)^{1-}$ should be the useful yield of ATP hydrolysis. This may include one or several elementary steps in which FeMoco or the P-cluster in Av1 gets prepared to accept the electron from Av2.

The energy profile of the transfer of one electron in nitrogenase is shown in Fig. 2. The E_m values for one-electron reduction of Av2 (complexed with MgATP) and P- and FeMoco-clusters in Av1 with dithionite are also given.[6,7,9,17] It can be seen that electron transfer giving super-reduced $(*FeMoco)^{1-}$ occurs against the redox potential at the expense of ATP hydrolysis energy. To estimate the redox potential of $(*FeMoco)^{1-}$, we took into account the facts that two ATP molecules are hydrolyzed per every transferred electron and that ΔF° for the hydrolysis of one ATP molecule equals -30.5 kJ mol^{-1} at 25 °C (pH 7.0). Then for the transfer of a single electron, the E_m value for the $FeMoco^N \rightleftarrows (*FeMoco)^{1-}$ transition in Av1 can be -0.67 V (-0.04 V + (-0.63 V)). In biological energy transformation systems, the coefficient of efficiency of ATP hydrolysis can reach 85%.[23] Assuming that the coefficient of efficiency of ATP hydrolysis in nitrogenase is no less than 85%, then E_m for this transition is about -0.6 V. If the transfer of the first electron from Av2 to Av1 is not followed by its further transfer on the substrate (as shown previously[13]), then the subsequent transfer of the second electron in a process coupled with hydrolysis of two MgATP molecules gives the super-reduced FeMoco cluster, $(*FeMoco)^{2-}$ (the transfer of the second electron is not shown in Fig. 2). No data on the E_m for the $(*FeMoco)^{1-} \rightleftarrows (*FeMoco)^{2-}$ transition have

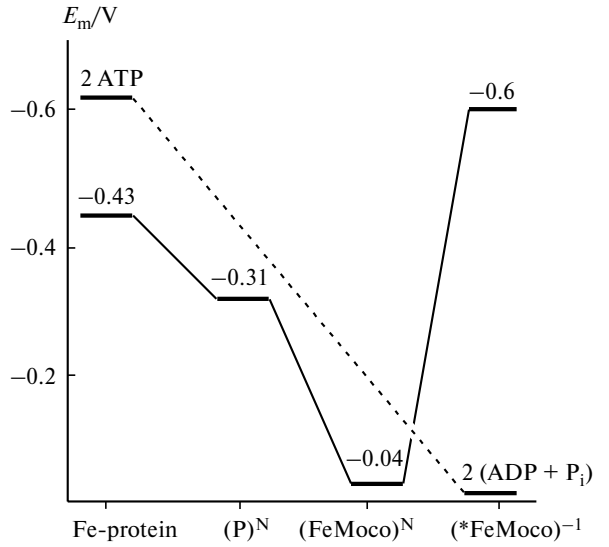

Fig. 2. Energy profile for the transfer of one electron in nitrogenase coupled with hydrolysis of two MgATP molecules.

been reported. A calculation similar to that performed for $(*FeMoco)^{1-}$ gives E_m for this transition of approximately −1 V.

If Av2 has been deliberately modified by site-specific mutagenesis (deletion of Leu 127 (L 127Δ)), the E_m value for the $[4Fe—4S]^{2+} \rightleftharpoons [4Fe—4S]^{1+}$ transition in Av2 complexed with Av1 becomes −0.62 V (see Ref. 29). One electron can be transported from Av2 to Av1 without MgATP, but at a lower rate than in the presence of MgATP. The second electron is not transferred without MgATP. In this case, the electron transfer cannot occur against the redox potential, as ATP is not hydrolyzed.

In the case of the nitrogenase complex $Av1 \cdot (Av2)_2$ stabilized by $MgATP \cdot AlF_4^-$, the E_m values for the [4Fe—4S] cluster in Av2, for the P-cluster and FeMoco in Av1 are −0.55, −0.43, and −0.09 V, respectively.[30] In the strong Cp2 complex with Av1, the E_m value for the [4Fe—4S] cluster of Cp2 decreases by 0.2 V (to −0.6 V).[31] In the Cp2 · Av1 complex, the rate constant for the electron transfer from Cp2 to Av1 without MgATP is 0.007 s^{-1}, while MgATP accelerates the electron transfer 10^4-fold.[31]

Calculations according to Marcus equation were carried out as follows:[31]

$$\log k_{et} = 15 - 0.6\,R - 3.1\,(\Delta G - \lambda)^2/\lambda,$$

where k_{et}/s^{-1} is the rate constant for electron transfer, R/Å is the distance between the donor and the acceptor, ΔG/eV is the free energy difference between the donor and the acceptor, λ/eV is the reorganization energy. The calculations showed that a 10^4-fold change in k_{et} requires a change in the potential ΔE_m for the donor and the acceptor of −0.62 to −0.72 V. Based on these data, it is clear that this ΔE_m value is not attained upon binding of MgATP to Av2. However, for hydrolysis of two MgATP molecules (see Fig. 2), ΔF° value corresponds to ΔE_m equal to −0.63 V. Hence, the thermodynamic profile of the ATPase reaction (see Fig. 2) can provide a 10^4-fold increase in k_{et}. The question of how this can occur has not been answered as yet.

Some stages (1—4, see Fig. 1) of ATP hydrolysis are matched by negative ΔF° values. These stages are related to the change in the protein conformation. Hence, this change should enable the processes that increase k_{et}. The acceleration of the electron transfer should be coupled with intermediate stages of MgATP hydrolysis and may be related to changes in ΔE_m and in the distance between the reaction participants, the formation of appropriately oriented electron and proton transfer paths, the change in the charge geometry, polarity of the environment of the Fe—S clusters, and so on. A certain role can be played by the phosphate-containing intermediate formed in ATP hydrolysis.

It follows from the foregoing that, despite the substantial progress in the studies of structure and redox properties of nitrogenase clusters, essential points in the nitrogenase functioning mechanism still remain obscure. The key point is as follows: how are eight electrons needed for nitrogen reduction on FeMoco accumulated and what is the role of P-clusters in this process? It is unknown how P^N accepts the electron(s). Whether is the ability of the P-cluster to exist in three oxidation states (P^+, P^{2+}, P^{3+}) related to the possibility of accumulation of electrons on the P-cluster for the subsequent multielectron reduction of nitrogen? Why is only one transition, $P^{2+} \rightleftharpoons P^+$, pH-dependent[8]?

In addition, it is important to find out what particular cluster is transformed to the super-reduced state. Obviously, this cluster should be located in the close vicinity of the ATPase center, as the electron transfer is coupled with ATP hydrolysis. We analyzed the structure of the nitrogenase complex $Av1 \cdot (Av2)_2$ reported previously.[32] A fragment of the structure (Fig. 3) was obtained using a database.[33] This complex has been stabilized[32] using $MgADP \cdot AlF_4^-$, which is bound to Av2 in the ATPase center.

We decided to elucidate the distances between the nitrogenase metal clusters and (1) ATP binding site, (2) ATP hydrolytic site, (3) amino acid residues able to be protonated (NH_2 groups of histidine and arginine).

Examination of the structure of the nitrogenase complex has shown that the ATP binding site and all nitrogenase metal clusters are located in one plane and the distance between each of these and the nearest partner is ~14—15 Å (see Fig. 3). Therefore, despite the fact that in the binding site, ATP decreases E_m of the [4Fe—4S] cluster of Av2 due to conformational changes, this takes place too far from the P- and FeMoco- clusters to affect them. The E_m value of the [4Fe—4S] cluster in Av2 is only −0.43 V, which is insufficient to perform the electron transfer against the potential.

Now we consider the groups located near the metal clusters that can be protonated. In Av2, all the four histidine residues occur on the protein surface at a distance of about 12—14 Å (not shown in Fig. 3), while the Arg α100, Arg β100, and Arg β140 residues, at a distance of about 10 Å from the [4Fe—4S] cluster (see Fig. 3). No lysine residues are found at distances less than 10 Å.

The P-cluster has three histidine residues, His α82, His β89, and His β192, four arginine residues, Arg α59, Arg α181, Arg α186, and Arg β99, and two lysine residues, Lys α67 and Lys β67, at distances of 8 to 10 Å.

The FeMoco cluster has His α441 at a 2.91 Å distance and two histidine residues, His α194 and His α273, at distances of 3.24 and 5.92 Å. Other histidine, lysine, and arginine residues at distances shorter than 10 Å are also present (these are not shown in Fig. 3): His 195, 361, 450 (all α), Arg 59, 95, 96, 276, 358, 360 (all α), Arg β104, and Lys 67 and 425 (both α).

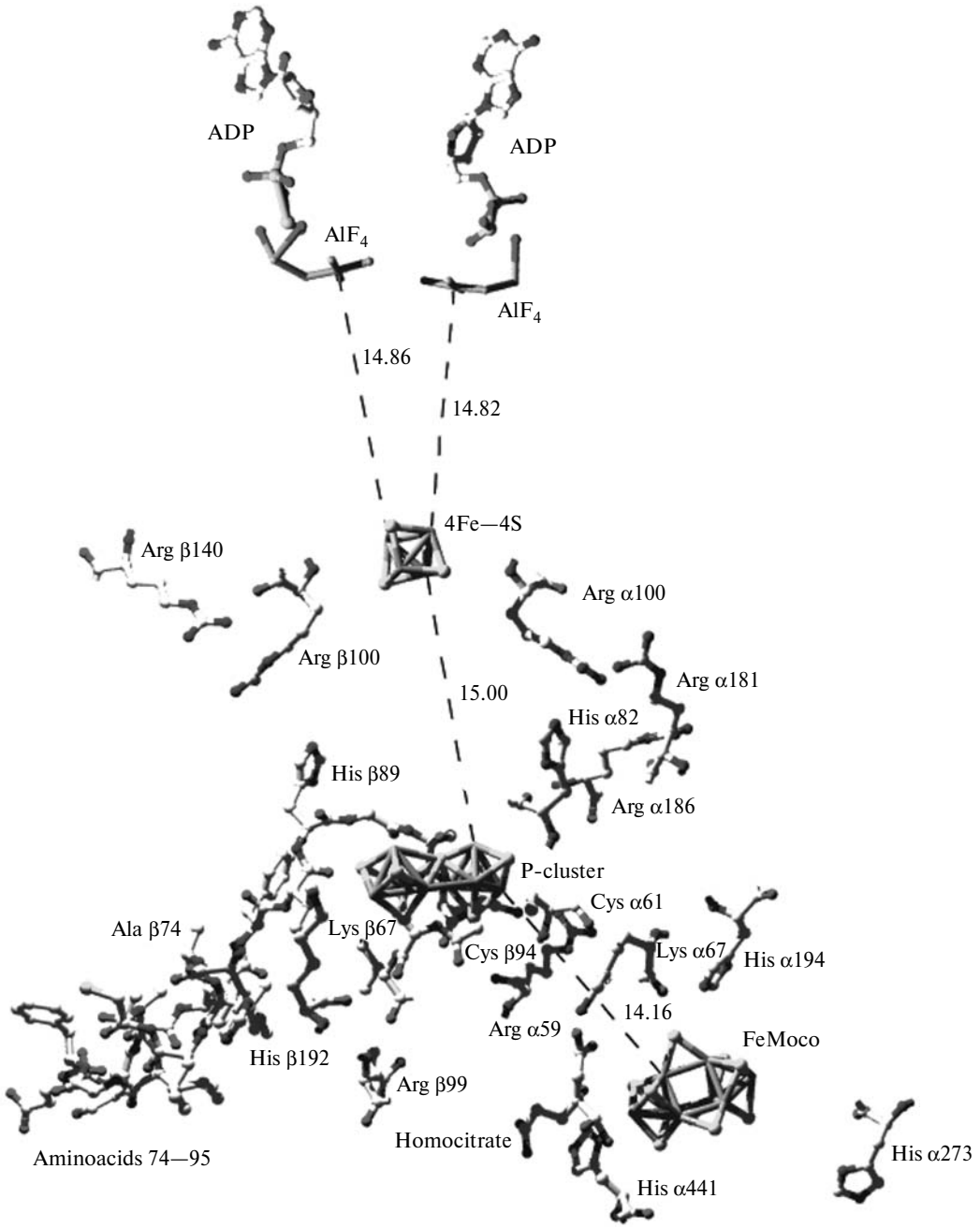

Fig. 3. Fragment Av1 · Av2 of the structure of nitrogenase complex Av1 · $(Av2)_2$ stabilized by $MgADP \cdot AlF_4^-$.[32,33] Dashed lines denoted the distances between the clusters in Å. Only amino acids having groups subject to protonation and nearest to the [4Fe—4S], P, and FeMoco clusters and amino acid sequence 74—95 are shown.

Thus, no amino acid groups that could be protonated and affect E_m of the [4Fe—4S]- or P-cluster are present in the close vicinity (less than 10 Å) of these clusters.

The hydrolytic ATPase site of nitrogenase is the key object of analysis presented in this communication. Previously,[34] it has been shown that ATP hydrolysis in nitrogenase gives no phosphoryl enzyme with a covalent type of bonding. It is known that, apart from the ATP binding site, ATPases contain a hydrolytic ATPase site. As a rule, these are different ATPase sites.[24] The hydrolytic ATPase site contains a carboxy group. During ATP hydrolysis in ATPases, this group plays a crucial role in the formation of the phosphate-containing intermediate representing a complex of the enzyme with $Mg^{2+}P_i$ and ADP, which are bound to the ATPase through the Mg^{2+} ion. This hydrolytic site catalyzes the direct ^{18}[O]-exchange between P_i and H_2O (see Ref. 23).

A comparison of the amino acid sequences of ATPases, a Fe-protein, and a Mo—Fe-protein from various microorganisms[35] indicates that the ATP binding site is located in the Fe-protein, while the hydrolytic ATPase site is on the Mo—Fe-protein. Indeed, Mo—Fe proteins, including Av1, contain, in the β-subunit, a highly conservative sequence of amino acids 74—95, which is characteristic of the hydrolytic ATPase site.

It has been reported[36] that Av1 has a weak affinity to ATP and ADP. This is due to the fact that it serves for the formation of the phosphate-containing intermediate. This conclusion is also supported by the following data. In nitrogenase, both protein components are needed for the hydrolysis of ATP. The Av2 protein does nor catalyze ATP hydrolysis. Presumably,[6] Av1 participates in the ATP hydrolysis. The ATPase activity of nitrogenase may be blocked by *o*-phthalaldehyde, which reacts with the NH_2 group of Av1 rather than Av2 (see Ref. 37). A good piece of evidence supporting the existence of this site has been found[38] in a study of so-called direct [^{18}O]-exchange between P_i and water. Av1 protein catalyzed this exchange, while ATP and ADP inhibited it. The Av2 protein did not catalyze the direct [^{18}O]-exchange between P_i and water. We suggested that there exist two functionally different ATPase sites in nitrogenase and that the hydrolytic ATPase site resides in Av1.

Of exceptional interest is the fact that amino acids 74—95 in Av1 "envelop" the P-cluster in Av1 (see Fig. 3) and occur at van der Waals distances from the P-cluster. Thus, one can suggest that the intermediate phosphate-containing nitrogenase—$Mg^{2+}P_i$ complex (intermediate of the ATPase reaction of nitrogenase) occurs in the close vicinity of the P-cluster and may be even coordinated to it. By enhancing the acceptor properties of the P-cluster, it can facilitate the electron transfer to the P-cluster from the Fe-protein against the potential. Therefore, by accepting an electron during ATP hydrolysis, the P-cluster might be converted into the super-reduced state.

Amino acids 74—95 in Av1 emerge on the protein surface. Thus Gln 82 and Lys 83 appear on the surface by at least 20%. In addition, there is a 140 Å^3 cavity located near the Av1 surface and contacting with the amino acids incorporated in the loop 74—95: Gln 92, Gly 43, Cys 94, Val 95. The size of this cavity is sufficient for the interaction with the phosphate intermediate. The accessibility of the cavity can change during ATP hydrolysis. A direct channel for water access (perhaps, also for other reagents) from the protein surface to the P-cluster and amino acid 74—95 loop, in particular, to Gln 92, also exists. However, it is unknown why the region of amino acids 74—95 in Av1 is located at such a great distance from the AlF_4^- binding site (see Fig. 3), which is considered[32] to reside in the ATP γ-phosphate binding site. Perhaps, in the native nitrogenase complex not stabilized *in vitro*, the hydrolytic site in Av1 is brought closer to the MgATP binding section in Av2 during MgATP hydrolysis than in the stabilized nitrogenase complex, $MgADP \cdot AlF_4^-$. Indeed, it has been shown[39] that the distance between the redox centers in nitrogenase, a key parameter determining the electron transfer rate in nitrogenase, depends on MgATP binding and hydrolysis processes. The Av1 protein contains a number of sites for binding Av2. During the formation of the active site for ATP hydrolysis, the section of interaction of Av2 and Av1 smoothes down. The [4Fe—4S] cluster of Av2 approaches the Av2 surface and becomes separated from the Av1 surface by the van der Waals distance. It was shown[39] that binding and hydrolysis of MgATP may be accompanied by significant migration of Av2. As a consequence, the amino acid residues located on the Av2 protein surface adjacent to Av1 can move by a distance of up to 20 Å.

Data indicating that P_i transfer from one amino acid to another may occur during ATP hydrolysis in ATPase have been reported.[40] No data of this type concerning nitrogenase are available. The intermediate steps of ATP hydrolysis have not been studied. Only the data on direct [^{18}O]-exchange between P_i and water do attest to the existence of a phosphate-containing intermediate.

In the $Av1 \cdot (Av2)_2$ structure, a chain of four water molecules is located between the P-cluster and FeMoco.[32] The first water molecule from the FeMoco side is linked to the homocitrate molecule by hydrogen bond. All the four water molecules in the chain are at hydrogen-bond distances from one another. From the P-cluster side, the fourth water molecule is bound by a hydrogen bond to Cys α61, which is a Fe(3) ligand of the P-cluster,[3] and to Cys β94, which is a part of the loop. This chain may serve for the transfer of both a proton and an electron from the P-cluster to FeMoco. Homocitrate is surrounded by six water molecules, which are bound by hydrogen bonds to one another and to homocitrate. They probably serve for proton accumulation in the substrate binding and reduction site. No chain of water molecules is located between the [4Fe—4S]-cluster of the Fe-protein and the P-cluster.

The above-outlined facts indicate that hydrolysis of MgATP can favor the electron transfer from Av2 to the P-cluster of Av1 and suggest that during the electron transfer from Av2 to Av1 coupled with ATP hydrolysis, it is the P-cluster that passes into the super-reduced state and then transfers an electron to FeMoco, thus transforming it also in the super-reduced state.

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