ISSN 0006-2979, Biochemistry (Moscow), 2010, Vol. 75, No. 1, pp. 81-84. © Pleiades Publishing, Ltd., 2010.
Published in Russian in Biokhimiya, 2010, Vol. 75, No. 1, pp. 99-104.

Role of Short Conserved Segments of α- and β-Subunits that Link F_1-ATPase Catalytic and Noncatalytic Sites

A. N. Malyan

Institute of Basic Biological Problems, Russian Academy of Sciences, ul. Institutskaya 2, 142290 Pushchino, Moscow Region, Russia; fax: (496) 733-0532; E-mail: malyan@ibbp.psn.ru

Received January 19, 2009
Revision received March 19, 2009

Abstract—An analysis of amino acid sequences and 3D structures of chloroplast, mitochondrial, and bacterial F_1-ATPases revealed that in their α- and β-chains there are short highly conserved segments linking in pairs the catalytic and noncatalytic sites. The analysis was based on the reported effect of directed mutagenesis of amino acids forming these segments on catalytic properties of the F_1-ATPases. It is proposed that one of these segments is responsible for transduction of a conformation signal from the noncatalytic to catalytic site upon ADP-for-ATP substitution at the noncatalytic site. At the catalytic site, this signal changes position of the terminal amino acid residue with respect to the adenine part of the molecule and results in a lower tightness of MgADP binding and its dissociation followed by enzyme activation. Mutagenesis of amino acids comprised by the two other segments was shown to produce an effect on the rate of cooperative catalysis, whereas the rate of single-site catalysis remained unaffected. This suggests that these segments are responsible for the cooperative mode of enzyme functioning.

DOI: 10.1134/S0006297910010104

Key words: coupling factor F_1, F_1-ATPase, ATP synthase, noncatalytic sites

ATP synthases of energy converting membranes of chloroplasts, mitochondria, and bacteria consist of a water-soluble peripheral part (F_1) and a membrane part (F_o). The former comprises 3 α-, 3 β-, 1 γ-, 1 δ-, and 1 ε-subunit. A minimum of the latter includes 1 *a*-, 2 *b*-, and 10 *c*-subunits [1]. At the interface between α- and β-subunits, there are three catalytic and three "noncatalytic" sites. The name of noncatalytic sites reflects an extremely low, catalysis-incompatible rate of nucleotide exchange at these sites. Both noncatalytic and catalytic sites are mostly formed by amino acids of α- and β-subunits, respectively [2]. In the course of ATP synthesis, the energy conversion is realized in two steps. First, a hydrophobic part of the ATP synthase complex (F_o) transfers protons across the membrane to convert the transmembrane proton potential into rotational energy of the *c*-subunits and their linked γ- and ε-subunits [3, 4]. Next, specific amino acids of the rotating γ-subunit interact successively with amino acids of each β-subunit to change conformation of the catalytic site, which results in ADP and phosphate binding followed by their conversion into dissociating ATP. Thus, at this step the mechanical energy of the "rotor" is converted into chemical high-energy bonds of ATP.

An important prerequisite to F_1-ATPase catalytic activity consists in coordinated (cooperative) functioning of the catalytic sites that allows using energy of reaction substrate binding to provide dissociation of the reaction products (the principle of energy recuperation [5-8]). A detailed scheme of cooperative catalysis involving the three catalytic sites and diagrams showing changes in the enzyme energy level during the course of the reaction are given in [9, 10]. The reaction velocity increases by 5-6 orders of magnitude in passing from single-site catalysis (that occurs at substrate/enzyme < 1) to cooperative catalysis [11, 12]. It is commonly supposed that coordination of separate reaction steps at the three catalytic sites is performed by the rotating γ-subunit. However, it should be noted that activities of subcomplexes with the γ-subunit ($\alpha_3\beta_3\gamma$) and without it ($\alpha_3\beta_3$) differ far less [13, 14]. This suggests that, apart from the γ-subunit, the interacting α- and β-subunits also contribute to cooperativity of the catalytic sites.

Membrane de-energization makes ATP hydrolysis coupled with transmembrane proton transportation thermodynamically possible. This reaction slows down with time due to tight MgADP binding to one of the catalytic sites [15, 16] followed by slow reversible inactivation of the enzyme [17-19]. The potency of tight MgADP binding,

and hence, that of enzyme inactivation, is substantially determined by asymmetric interaction between the γ-subunit and the β-subunits. Enzymes lacking the γ-subunit showed no MgADP-induced inactivation [20]. On the other hand, experiments on F_1 from chloroplasts, mitochondria, and bacteria [21-29], as well as on chloroplast ATP synthase [30-33], proved that ATP and/or oxyanion binding to noncatalytic sites resulted in a lower tightness of MgADP binding, and accordingly, in a lower inactivation level. A certain influence of catalytic sites on noncatalytic ones was also reported: given MgADP-dependent enzyme inactivation, a somewhat lower rate of noncatalytic site filling with nucleotides was observed [25]. This is evidence for existence of an F_1-ATPase regulatory system that includes the noncatalytic sites and is capable of modulating the MgADP-dependent inactivation of the enzyme.

To date, a few attempts to elucidate the mechanism of the relationship between the catalytic and noncatalytic sites have been made. Allison's team studied the effect of point mutations made within a cluster of hydrophobic amino acid residues at the interface between the α- and β-subunits of F_1-ATPase from the thermophilic bacterium *Bacillus* PS3 [34]. As shown, mutation of each of three interacting residues (αF244C, αR304C, and αY300C) caused a change in excess magnesium-dependent degree of ATPase activity inhibition, and hence, a different level of MgADP-induced inactivation of the enzyme. These residues were supposedly localized on the signal transduction pathway between the catalytic and noncatalytic sites. It is significant that the enzyme activity showed mutation dependence even without any sign of the inactivating effect of MgADP, that is, at the initial moment of the reaction or in the presence of lauryl dimethylaminoxide. This indicates that the effect of these mutations is most probably unspecific. Richter's team studied the effect of mutations at the C-terminal part of the γ-subunit of chloroplast F_1 (CF_1) reconstituted with the F_1 α- and β-subunits of the photosynthesizing bacterium *Rhodospirillum rubrum* [35]. The involvement of the mutated residues in conformation signal transduction between the catalytic and noncatalytic sites was judged from the sulfite oxyanion effect on ATPase activity of the hybrid F_1. This approach was supported by reports that the stimulating effect of oxyanions was caused by their interaction with the ATP γ-phosphate binding site at the noncatalytic site [29]. It was found that substitution of γGlu305 for Ala resulted in complete enzyme insensitivity to the sulfite effect. This might be indicative of disturbance of the conformation signal transduction from the noncatalytic to catalytic site. However, in case of similar mutation at the γ-subunit reconstituted with the α- and β-subunits of chloroplast F_1, the activating effect of sulfite was preserved [36]. Mutation of the adjacent residues did not affect the sensitivity to sulfite either.

The current search for a molecular segment responsible for transduction of the conformation signal between catalytic and noncatalytic sites was based on supposition that such a segment should comprise amino acid residues pertaining to the sites in question, because even minor deviations in residue coordinates result in activity fluctuation. To find the coordinates, the Brookhaven Databank and the program RasMol (R. Sayle (1994)) were used. Also, the protein molecule was considered as a system of elements with isolated degrees of freedom that can perform transduction of the conformation signal within the molecule without any energy loss, on the same principle as a wrapped flexible cable [5, 6]. Since nothing was known about amino acids involved in this transduction performing segment, a prerequisite to the minimum in energy loss was the minimal length of the segment. This requirement is met by inner protein segments the length of which is close to the minimal distance between the catalytic and noncatalytic sites. For mitochondrial F_1, this distance is 27 Å [2]. Hence, with an average length of one polypeptide chain unit of 3.6 Å [37], a segment linking adjacent nucleotide binding sites has to comprise at least eight amino acids or more, with density of protein folding taken into account. As follows from our analysis, one of these segments (designated as M1) is that of the β-subunit linking Tyr345 with Arg356 (herein, numbers of the sequence of mitochondrial coupling factor F_1 are used). Since within this segment the sequences of the α- and β-subunits from coupling factors of different origin are virtually homologous, it is believed to be conserved (Fig. 1). Segment M1 meets the requirement of energy conservation in the course of signal transduction from its one end to the other [5]: no residue of this segment, except Asp352, is involved in Coulomb interaction with surrounding residues, while Asp352 through its interaction with Arg171 can pass the signal to Gln172 that is localized, together with Arg356, near the phosphate end of the nucleotide at the same noncatalytic site. Tyr345 belongs to the catalytic site [2], and the distance between this residue and a purine part of the adenine nucleotide is less than 3.5 Å (Fig. 2). Arg356, a member of the noncatalytic site, is almost the same distance away from the ATP γ-phosphate. Tyr345 is believed to provide the hydrophobic environment of adenine [38, 39]; the energy of its interaction with ATP is 1.5 kcal/mol [39]. An increase in hydrophobicity of the environment achieved through Tyr-for-Phe substitution caused a decreased MgADP-dependent inactivation of the enzyme [38]. The inactivating effect was still weaker with ATP substitution for ε-ATP that shows a higher hydrophobicity [40]. When assessing Tyr345 substitution dependence of the catalytic activity, its dependence on the residue size (10-fold affinity decrease in the order of Tyr > Leu > Ala) was also noticed [41]. The effect of Arg356 substitution on other residues was not studied. Interestingly, the residue αArg373, homologous to Arg356 and similarly localized, although at the catalytic site, showed a pronounced mobility and shifted by 1.5 Å when ADP-for-ATP substi-

	CS	NS
β - spinach chloroplasts	Y(362) PAVDPLDSTS	T(373)
β - Sy6301	Y(352) PAVDPLDSTS	T(363)
β - bovine heart mitochondria	Y(345) PAVDPLDSTS	R(356)
β - *E. coli*	Y(331) PAVDPLDSTS	R(342)

	CS	..NS..	CS
α - spinach chloroplasts	S(336) ITDGQIFL	SADLFNAGIRPAINVGISVSR	(365)
α - Sy6301	S(336) ITDGQIFL	SSDLFNSGLRPAINVGISVSR	(365)
α - bovine heart mitochondria	S(344) ITDGQIFL	ETELFYKGIRPAINVGLSVSR	(373)
α - *E. coli*	S(347) ITDGQIFL	ETNLFNAGIRPAVNPGISVSR	(376)

Fig. 1. Amino acid sequences linking a catalytic site (CS) with a noncatalytic site (NS). Conserved residues are shown in black.

tution took place [39]. Since in chloroplast F_1 Thr occupies the position of Arg, most probably its interaction with terminal phosphates is realized through H-bonds.

The proposed mechanism allows the following explanation of the activating effect of ATP and oxyanions. ATP binding to the noncatalytic site accompanied by appearance of the γ-phosphate presumably causes a shift in the position of Arg356. This motion conferred to Tyr345 via the sequence Ser355-Pro346 and accompanied by changing hydrophobic interactions with the purine part of ADP tightly bound at the catalytic site results in weakening of ADP binding and thereby initiates the enzyme transition from its inactive to active state. A similar effect can be produced by oxyanions that bind to the noncatalytic site in the position of the γ-phosphate [29].

The other two segments linking noncatalytic sites with their adjacent catalytic sites belong to the α-subunit. As seen from Fig. 1, these are conserved as well. Segment M2 begins at Phe357 near the ATP ribose ring of the noncatalytic site and ends at Ser344 near the ATP γ-phosphate of the catalytic site (Fig. 3). The other segment (M3) links Arg362 and Pro363 positioned near ATP adenine of the noncatalytic site with Arg373 which, though uninvolved directly in the catalytic cycle, is responsible for coordinated (cooperative) functioning of the three catalytic sites [42]. These segments comprise the following residues the effect of which on bacterial F_1 properties was studied using point mutation: αS347F, αG351D and αS373F, αS375F [39, 43, 44]. Their corresponding residues are S344, G348 from one segment and S370, S372 from the other segment of the mitochondrial F_1 α-subunit (Fig. 3). It should be noted that mutation of these residues, in spite of difference in their position and origin, gave results of the same type. All mutations used, as well as Arg373 substitutions, resulted in a change in positive catalytic cooperativity accompanied by a minor effect on nucleotide binding [43, 44]. The common feature consisted in the fact that the introduced residue was much larger than the replaced one. Besides, the observed effects are in good agreement with our notion of the mechanical character of conformation signal transduction: in the sequence under study any residue of a larger size hampers the progress of the entire segment of the polypeptide chain relative to its surroundings, and hence, affects relative positions of the terminal residue and its closest nucleotide site. Like segment M1, segments M2 and M3 link in pairs neighboring catalytic and noncatalytic sites (Fig. 3).

The linking scheme is in good agreement with experimental results showing that modification of one noncatalytic site decreases the ability of ATP to keep F_1 in its active state by 1/3, modification of two sites gives a decrease by 2/3, and modification of all of them results in virtually full inactivation of the enzyme [45] (see Scheme). However, it can be believed that segments M2 and M3 are

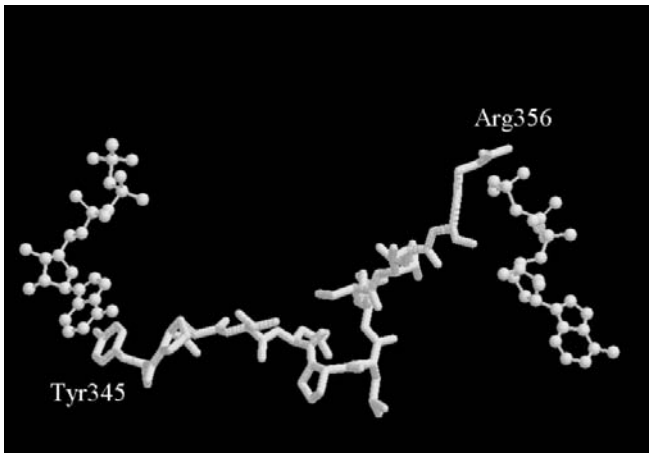


Fig. 2. Amino acid sequence of segment M1 (βTyr345-βArg356) linking a catalytic site with a noncatalytic site (the spheres are pictorial rendition of ATP atoms).

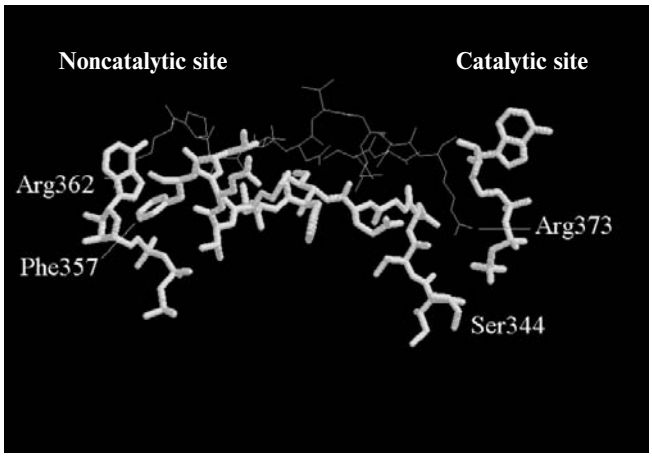
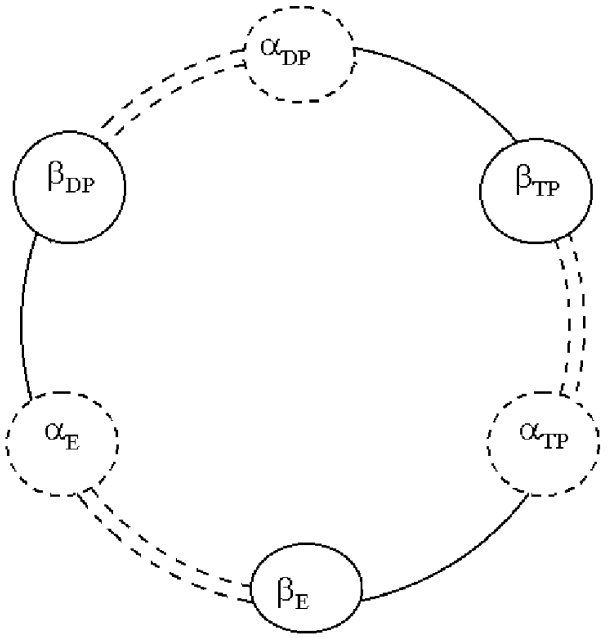


Fig. 3. Amino acid sequences of segment M2 (αSer344-αPhe357) and segment M3 (αArg362-αArg373) (shown as thin lines) linking a catalytic site with a noncatalytic site.



A scheme of linking of catalytic and noncatalytic sites of F_1. Segments M1 are shown as solid lines, segments M2 and M3 are shown as dashed lines. The subunits are denoted according to [2]

responsible for consecutive changing of conformation of the three catalytic sites that is strictly coordinated with rotation of the γ-subunit and required to maintain high velocity of the cooperative catalysis reaction.

This study was supported by the Russian Foundation for Basic Research (No. 06-04-49315a).

REFERENCES

1. Jiang, W., Hermolin, J., and Fillingame, R. H. (2001) *Proc. Natl. Acad. Sci. USA*, **98**, 4966-4971.
2. Abrahams, J. P., Leslie, A. G. W., Lutter, R., and Walker, J. E. (1994) *Nature*, **370**, 621-628.
3. Boyer, P. D. (1993) *Biochim. Biophys. Acta*, **1140**, 215-250.
4. Senior, A. E., Nadanaciva, S., and Weber, J. (2002) *Biochim. Biophys. Acta*, **1553**, 188-211.
5. Khurgin, J. I., Chernavsky, D. S., and Shnoll, S. E. (1967) *Mol. Biol. (Moscow)*, **1**, 419-423.
6. Blumenfeld, L. A. (1977) *Problems of Biological Physics* [in Russian], Nauka, Moscow.
7. Repke, K. R. H., and Schon, R. (1974) *Acta. Biol. Med. Germ.*, **33**, K27-K38.
8. Kayalar, C., Rosing, J., and Boyer, P. D. (1977) *J. Biol. Chem.*, **252**, 2486-2491.
9. Weber, J., and Senior, A. E. (2003) *FEBS Lett.*, **545**, 61-70.
10. Tikhonov, A. N., Pogrebnaya, A. F., and Romanovsky, J. M. (2003) *Biofizka*, **48**, 1052-1070.
11. Grubmeyer, C., and Penefsky, H. S. (1981) *J. Biol. Chem.*, **256**, 3728-3734.
12. Dunkan, T. M., and Senior, A. E. (1985) *J. Biol. Chem.*, **260**, 4901-4907.
13. Al-Shawi, M. K., Parsonage, D., and Senior, A. E. (1990) *J. Biol. Chem.*, **265**, 5595-5601.
14. Gromet-Elhanan, Z., and Avital, S. (1992) *Biochim. Biophys. Acta*, **1102**, 379-385.
15. Smith, L. T., Rosen, G., and Boyer, P. D. (1983) *J. Biol. Chem.*, **258**, 10887-10894.
16. Drobinskaya, I. Y., Kozlov, I. A., Murataliev, M. B., and Vulfson, E. N. (1985) *FEBS Lett.*, **182**, 419-424.
17. Vasilyeva, E. A., Minkov, I. B., Fitin, A. F., and Vinogradov, A. D. (1982) *Biochem. J.*, **202**, 9-14.
18. Malyan, A. N., and Vitseva, O. I. (1983) *Biokhimiya*, **48**, 718-724.
19. Malyan, A. N., and Vitseva, O. I. (1990) *Photosynthetica*, **24**, 613-622.
20. Sokolov, M., and Gromet-Elhanan, Z. (1996) *Biochemistry*, **35**, 1242-1248.
21. Milgrom, Y. M., Ehler, L. L., and Boyer, P. D. (1990) *J. Biol. Chem.*, **265**, 18725-18728.
22. Milgrom, Y. M., Ehler, L. L., and Boyer, P. D. (1991) *J. Biol. Chem.*, **266**, 11551-11558.
23. Murataliev, M. B., and Boyer, P. D. (1992) *Eur. J. Biochem.*, **209**, 681-687.
24. Jault, J. M., and Allison, W. S. (1993) *J. Biol. Chem.*, **268**, 1558-1566.
25. Milgrom, Y. M., and Cross, R. L. (1993) *J. Biol. Chem.*, **268**, 23179-23185.
26. Matsui, T., Muneyuki, E., Honda, M., Allison, W. S., Dou, C., and Yoshida, M. (1997) *J. Biol. Chem.*, **272**, 8215-8221.
27. Malyan, A. N., and Allison, W. S. (2002) *Biochim. Biophys. Acta*, **1554**, 153-158.
28. Malyan, A. N., and Vitseva, O. I. (2001) *Biochemistry (Moscow)*, **66**, 410-414.
29. Malyan, A. N. (2003) *Biochim. Biophys. Acta*, **1607**, 161-166.
30. Malyan, A. N. (2005) *Biochemistry (Moscow)*, **70**, 1245-1250.
31. Malyan, A. N. (2006) *Photosynth. Res.*, **88**, 9-18.
32. Malyan, A. N. (2007) *Biochemistry (Moscow)*, **72**, 728-734.
33. Pronin, A. S., and Malyan, A. N. (2009) *Biochemistry (Moscow)*, **74**, 775-780.
34. Grodsky, N. B., Dou, C., and Allison, W. S. (1998) *Biochemistry*, **37**, 1007-1014.
35. He, F., Samra, H. S., Tucker, W. C., Mayans, D. R., Hoang, E., Gromet-Elhanan, Z., and Berrie, C. L. (2007) *Biochemistry*, **46**, 2411-2418.
36. He, F., Samra, H. S., Johnson, E. A., Degner, N. R., McCarty, R. E., and Richter, M. L. (2008) *Biochemistry*, **47**, 836-844.
37. Lehninger, A. L. (1976) *Biochemistry* [Russian translation], Mir, Moscow.
38. Wise, J. G. (1990) *J. Biol. Chem.*, **265**, 10403-10409.
39. Weber, J., and Senior, A. E. (1997) *Biochim. Biophys. Acta*, **1319**, 19-58.
40. Schaefer, H.-J., Mueller, H. W., and Dose, K. (1980) *Biochem. Biophys. Res. Commun.*, **95**, 1113-1118.
41. Weber, J., Lee, R. S., Grell, E., and Senior, A. E. (1992) *J. Biol. Chem.*, **267**, 1712-1718.
42. Le, N. P., Omote, H., Wada, Y., Al-Shawi, M. K., Nakamoto, R. K., and Futai, M. (2000) *Biochemistry*, **39**, 2778-2783.
43. Kanazawa, H., Noumi, T., Matsuoka, I., Hirata, T., and Futai, M. (1984) *Arch. Biochem. Biophys.*, **228**, 258-269.
44. Wise, J. G., Latchney, L. R., Ferguson, A. M., and Senior, A. E. (1984) *Biochemistry*, **23**, 1426-1432.
45. Amano, T., Matsui, T., Muneyuki, E., Noji, H., Hara, K., Yoshida, M., and Hisabori, T. (1999) *Biochem. J.*, **343**, 135-138.