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MONOCLONAL ANTIBODIES TO MITOCHONDRIAL F1-ATPase AND OLIGOMYCIN SENSITIVITY CONFERRING PROTEIN (OSCP). TOOLS FOR RECOGNITION OF WELL CONSERVED AND ESSENTIAL ANTIGENIC SITES

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SUMMARY : The preparation of anti-OSCP monoclonal antibodies is described for the first time. One of these antibodies prevents the activating effect of OSCP in reconstitution experiments. These antibodies and antibodies previously obtained against the α- and β-subunits of pig heart mitochondrial F1-ATPase have been used to look for well conserved epitopes in various species. One anti-β antibody can recognize all species tested while the anti-OSCP antibodies only recognize the pig or beef enzyme. The above anti-β antibody inhibits ATP synthesis without modifying the rate of ATP hydrolysis. This antibody also prevents the ADP-induced hysteretic inhibition of F1-ATPase.

Living cells synthesize most of their ATP through a transmembrane ATPase-ATPsynthase complex (OS-ATPase) made up of about 7 to 12 different polypeptides. The role, the assembly and the stoicheiometry of these polypeptides are still controversial (1). However two main parts have been described : the F1 and the FO or membrane sector. F1 can be purified as a water soluble ATPase and is made up of 5 subunits α, β, γ, δ, and ε in the mitochondrial system (2). The hydrolytic sites are reputed to be located on the β-subunits may be at the interface α/β (1). The FO part is not as well-defined (3). OSCP (oligomycin sensitivity conferring protein) is compulsory for the oligomycin-sensitive association of F1 to the membrane sector (4). In the present work, monoclonal antibodies prepared against the α and β subunits of F1 and against OSCP of the pig heart complex have been used as probes to differentiate well conserved antigenic sites from variant ones along the phylogenic scale. One antibody recognizes the β-subunit in all species tested, it partially prevents the hysteretic inhibition of ATPase activity due to ADP binding to regulatory site (5) and inhibits ATPsynthesis in the membrane, while hydrolytic sites are not affected. An anti-OSCP antibody specific to the pig or beef species prevents the

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activating effect of OSCP on the reconstitution of the ATP-synthase activity by reassociation of F1 to F1- and OSCP- depleted submitochondrial particles.

MATERIALS AND METHODS

Previously described procedures were used to obtain the various fractions from pig heart mitochondria (6), urea treated submitochondrial particles (ETP-U) (7), submitochondrial particles (8) from mitoplasts (9), F1 (10),OS-ATPase and OSCP by adaptation of the methods of Serrano et al (11) and Senior (12) respectively. Beef heart complex V was prepared according to Stigall et al (13). Spinach chloroplasts were prepared with the method of Binder et al (14). Rat liver mitochondria were kindly prepared by Dr. Baggetto following Schneider (15). Mitochondrial preparations of S. cerevisiae and A. niger were gifts of Drs. Somlo and Letoublon respectively. E. coli membrane fractions were from Dr. Manaï. F1 purified from E. coli was a generous gift of Prof. Hanstein These cooperations are gratefully acknowledged.

Protein concentration (16), ATPase activity and hysteretic inhibition (5) net ATP synthesis (17), ATP-^{32}Pi exchange and [γ -^{32}P]ATP hydrolysis (8) were as previously described. NaDodSO4 polyacrylamide (14 %) gel electrophoresis was performed according to Laemmli (18). The transfer of subunits onto nitrocellulose sheets was by the method of Towbin et al (19). The immune complex detection and preparation of monoclonal antibodies against α and β subunits of F1 were as previously reported (20).

Monoclonal antibodies against OSCP resulted from a fusion of SP2-0 myeloma cell lines with spleen cells from a Balb/c mouse immunized with pig heart OS-ATPase complex. Female mice, 4-6 weeks old, received several intraperitoneal injections : first with 200 µg of OS-ATPase complex in 100 µl PBS [10mM phosphate buffer (Na), 0.9 % NaCl, pH 7.2] emulsified with an equal volume of complete Freund's adjuvant. Then every fortnight 200 µg of complex in 200 µl PBS were injected. Three days after the fourth injection, mice were sacrified and spleens removed for cell fusion as described by Köhler and Milstein [21]. The positive clones were selected by solid-phase radioimmunoassay using F1 and OS-ATPase complex adsorbed on Terasaki plates (20). Antibodies were purified following Ey et al (22).

RESULTS AND DISCUSSION

1 - Detection of well conserved antigenic sites from the variant ones in the ATPase-ATPsynthase complex.

Three anti-β subunit and one anti-α subunit monoclonal antibodies have been prepared (20) after immunisation against the pig heart F1. Two of them 5G11 and 19D3 had a much stronger reaction with the active conformation of F1 than with depolymerized F1. Table I shows that 5G11 was the only one to cross react with the β-subunits of all species studied while 19D3 reacted with all but may be A. niger. In the latter case, a very faint reaction has been observed. Therefore a clear distinction between a positive or a negative reaction cannot be made until F1 can be purified from A. niger. This means that some

Table I - Recognition of antigenic sites of[a] different species by monoclonal antibodies prepared against the pig heart mitochondrial ATPase-ATPsynthase

Clone	Subunit[b] recognized	Pig heart OS-ATPase	Beef heart complex V	Rat liver mitochondria	A. Niger mitochondria	S. cerevisiae mitochondria	Spinach chloroplasts	E. Coli F1 or membranes
5G11	β	+	+	+	+	+	+	+
19D3	β	+	+	+	(±)	+	+	+
14D5	β	+	+	+	-	-	-	-
20D6	α	+	+	+	-	-	-	-
2B1B1	OSCP	+	+	-	-	-	-	-
5D6D1	OSCP	+	+	-	-	-	-	-

[a] The signs + and - respectively indicate recognition or absence of recognition of the corresponding subunit in each species by the monoclonal antibody tested. The parenthesis means that a very faint reaction has been observed.

[b] The specificity of recognition was tested as described in Moradi-Améli et al (20) except that the subunits were separated by polyacrylamide (14 %) gel electrophoresis performed in the presence of NaDodSO4 (18).

parts essential for the active conformation of F1 are well conserved over the phylogenic scale. This result is in agreement with the homology of about 70 % found between the primary sequences of β subunits of F1-ATPase from beef, E. coli (23) and chloroplasts (24). The anti-β 14D5 and anti-α 20D6 only recognized mammalian species.

Two anti-OSCP monoclonal antibodies were recently obtained. Fig. 1 demonstrates that 2B1B1 and 5D6D1 react only with a peptide of OS-ATPase that migrates as OSCP. Since identical autoradiograms were obtained with either purified OSCP or intact mitochondria, it can be concluded that these monoclonal antibodies are specific to OSCP. Besides, we see in Table I that the two anti OSCP antibodies against the pig heart protein only cross-react with beef heart OSCP. Therefore OSCP appears more specific to a species than the F1 subunits, although an homology of sequences has been reported for OSCP from beef heart and the δ subunit of E. coli (26). It should be added that a polyclonal antibody raised against the pig heart OSCP did not cross react with the δ subunit of E. coli either.

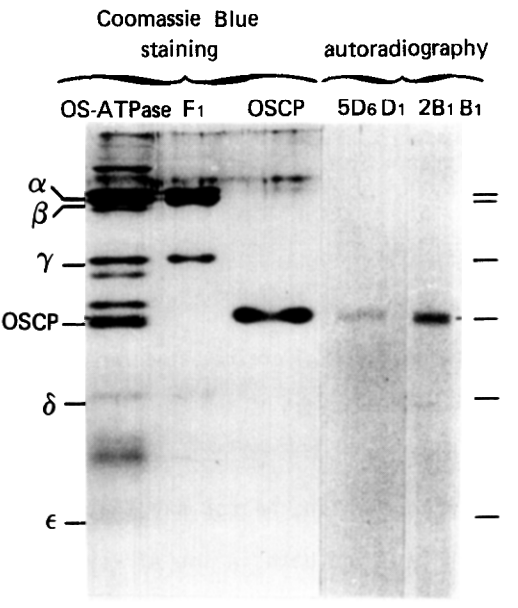

Figure 1 : Recognition of OSCP by the monoclonal antibodies 2B1B1 and 5D6D1. The peptides were separated by NaDodSO4 polyacrylamide (14%) gel electrophoresis according to Laemmli (18) using 20 µg of OS-ATPase or F1 and 8 µg of OSCP. The gels were either stained with Coomassie blue or transferred on nitrocellulose sheets (19). The autoradiography was performed after incubating successively the nitrocellulose with the antibody (culture supernatant), with rabbit anti-mouse immunologlobulin and with ^{125}I-protein A as previously described in (20). The autoradiography shown here was made after transfer of OS-ATPase to nitrocellulose.

Whether or not OSCP was denatured by urea-NaDodSO4, its reactivity with the anti-OSCP antibodies was the same. This means that the antibodies are specific to a sequence rather than to a conformation.

2 - Action of one anti-β antibody on the ATPase-ATPsynthase complex.

Since 5G11 recognizes a determinant on the β subunit common to all species, it appeared interesting to study its effects on the complex. Table II shows that 1.1 µM 5G11 inhibits net ATP synhtesis or ATP^{32}Pi exchange by 30 to 55 % in submitochondrial particles prepared from mitoplasts, while under the same conditions the hydrolysis of ATP was not inhibited. In fig. 2, we see that preincubation of F1 (0.5 µM) with 5G11 (0.9 µM) does not effect its rate of ATP hydrolysis either. In contrast, the binding of 5G11 to F1 partially prevents the ADP-induced hysteretic inhibition of the ATPase activity (5) and therefore prevents the conformational change responsible for the hysteretic

Table II - Effects of 5G11 on ATP synthesis and hydrolysis in submitochondrial particles

	Net ATP synthesis[a]	ATP-^{32}Pi exchange	[γ-^{32}P]ATP hydrolysis [a]
Control	340	247	5080
5G11[b]	151	173	5730

[a] Activity expressed as nmole ATP synthesized or hydrolyzed/min/mg protein. Net ATPsynthesis (17), ATP-^{32}Pi exchange and [γ-^{32}P]ATP hydrolysis (8) were as previously described.

[b] Submitochondrial particles (150 µg) preincubated for 1 h at 30° C with purified antibody 5G11 (1.1 µM, final concentration) in 150 µl of 0.14M Na-phosphate pH 7.5. The molecular weight of the IgG was taken as 150,000.

inhibition of F1 (25). In conclusion, we can say that anti-β antibody 5G11 recognizes a conformation involved both in the ATP-synthase activity and in the regulation of F1 by ADP. This conformation does not appear to be related to the ATP hydrolytic activity.

3 - Action of the anti-OSCP 2B1B1 antibody on the reconstitution of ATP synthesis in F1-depleted particles.

Table III shows that our preparations of urea treated submitochondrial particles (ETP-U) are well depleted from active F1 since the ATP synthesis is

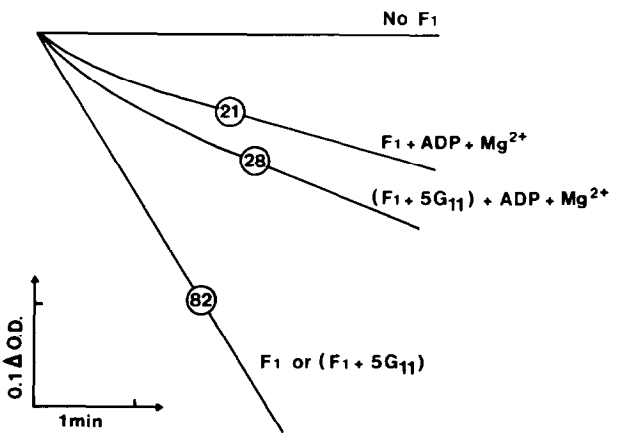

Figure 2 : Recordings of the rate of ATP hydrolysis after preincubation of F1 in the presence and absence of ADP + Mg^{2+} and 5G11. F1 (0.5 µM) was preincubated with 5G11 (0.9 µM) in 0.14 M Na-phosphate, pH 7.5 for 1 hr at 30°C or alone in the same buffer. The ATP hydrolysis was measured with a regenerating system as in Di Pietro et al (5). Hysteretic inhibition was recorded after a further preincubation of F1 alone or (F1 + 5G11) for 10 min with 0.2 mM ADP and 1.5 mM Mg SO4. Values indicate the activity expressed as µmoles ATP hydrolyzed/min/mg protein present as F1.

Table III - Effect of 2B1B1 on the reconstitution of ATP synthesis from depleted submitochondrial particles, F1 and OSCP

Preincubation conditions	ATP synthesis[a]	% inhibition induced by 2B1B1
ETP	350	
ETP + 2B1-B1	330	5 %
ETP-U	1-2	
ETP-U + F1	23	
ETP-U + OSCP + F1	50	
(ETP-U + 2 B1B1) + OSCP + F1[b]	50	0 %
ETP-U + (2B1B1 + OSCP) + F1[b]	25	96 %

The particles (ETP or ETP-U) were preincubated for 30 min at 30°C at a concentration of 0.3 mg protein/ml of buffer containing 5 mM ADP, 6 mM $MgCl_2$, 50 mM glucose, 30 hexokinase units (Sigma, type F 300), 0.25 M sucrose, 10 mM Tris, 20 mM Pi, 5 µCi ^{32}Pi, pH 7.5 in the presence or absence of the purified monoclonal antibody 2B1B1 (1 µM). OSCP (1 µg) and F1 (60 µg) were then added.The net ATP synthesis was initiated ten min later by adding 10 mM succinate and stopped after 4 min by adding 4 % perchloric acid.

[a] Net ATP synthesis (expressed as nmoles ATP formed/min/mg protein present as ETP or ETP-U) was measured as described in (17).

[b] The components in parenthesis were preincubated together for 30 min before addition of the other components.

almost nil. Reassociation with F1 increases ATPsynthesis from 1.2 to 23 nmol ATP/min/mg protein, but in the presence of 1 µg OSCP, the rate reached 50 nmol ATP/min/mg protein. The OSCP concentration chosen was about three times lower than the one giving the optimal activation of ATP synthesis in order to ensure a complete complexation of OSCP with the purified antibody during the preincubation time used in these experiments.

The antibody 2B1B1 had no effect on the succinate-dependent net ATP synthesis of ETP or of ETP-depleted from F1 and OSCP by urea treatment and reconstituted with F1 and OSCP except if the antibody was preincubated with OSCP before the reconstitution.

This antibody binds to ETP or to purified FO-F1 complex as demonstrated by solid-phase or competitive radioimmunoassays as well as by electronmicroscopy (not shown here). Therefore, the epitope recognized by this antibody is

accessible from the surface of the inner mitochondrial membrane. However, the binding of the antibody to this epitope does not interfere with ATP synthesis or proton translocation in functional ETP. When 2 B1B1 is preincubated with purified OSCP, it prevents the activating effect of OSCP on the reconstitution of ATPsynthesis. In this case, the antibody may modify the conformation of OSCP and prevent an efficient reconstitution or hinder the interaction between OSCP and either FO or F1. The identification of the epitopes recognized by the anti α and anti OSCP antibodies will enable us to give precise information on the organization of the functional complex.

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REFERENCES

1 - Senior, A.E. and Wise, J.G. (1983) J. Membrane Biol. 13, 105-124.
2 - Pullman, M.E., Penefsky, H.S., Datta, A. and Racker, E. (1960) J. Biol. Chem. 235, 3322-3329.
3 - Fillingame, R.H. (1980) Annu. Rev. Biochem. 49, 1079-1113.
4 - Mac Lennan, D.H. and Tzagoloff A. (1968) Biochemistry 7, 1603-1610.
5 - Di Pietro, A., Penin, F., Godinot C. and Gautheron, D.C. (1980) Biochemistry 19, 5671-5678.
6 - Godinot, C., Vial, C., Font, B. and Gautheron, D.C. (1969) Eur. J. Biochem. 8, 385-394.
7 - Horstman, L.L. and Racker, E. (1967) J. Biol. Chem. 242, 2547-2551.
8 - Penin F., Godinot, C., Comte J. and Gautheron, D.C. (1982) Biochim. Biophys. Acta 679, 198-209.
9 - Maïsterrena, B. Comte, J. and Gautheron, D.C. (1974) Biochim. Biophys. Acta 367, 115-126.
10 - Penin, F., Godinot, C. and Gautheron, D.C. (1979) Biochim. Biophys. Acta 548, 63-71.
11 - Serrano, R., Kanner, B.I. and Racker, E. (1976) J. Biol. Chem. 251, 2453-2461.
12 - Senior, A.E. (1979) Methods Enzymol. 55, 391-397.
13 - Stiggall, D.L., Galante, Y.M. and Hatefi, Y. (1978) J. Biol. Chem. 253, 956-964.
14 - Binder, A, Jagendorf, A. and Ngo, E. (1978) J. Biol. Chem. 253, 3094-3100
15 - Schneider, W.C. and Hogeboom, G.H. (1949) J. Nat. Cancer 10, 969-976.
16 - Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, J. (1951) J. Biol. Chem. 193, 265-275.
17 - Deléage, G., Penin, F., Godinot, C. and Gautheron, D.C. (1983) Biochim. Biophys. Acta 725, 464-471.
18 - Laemmli, H.K. (1970) Nature, 222, 680-685.
19 - Towbin, H., Staehlin, T. and Gordon, J. (1979) Proc. Natl Acad. Sci. USA, 76, 4350-4354
20 - Moradi-Améli, M. and Godinot, C. (1983) Proc. Natl. Acad. Sci. USA, 80, 6167-6171.

21 - Köhler G. and Milstein, C. (1975) Nature (London) 256, 495-497.
22 - Ey, P.L., Prowse, S.J. and Jenkin, C.R. (1978) Immunochemistry, 15, 429-436.
23 - Walker, J.E., Runswick, M.J. and Saraste M. (1982) FEBS Lett., 146, 393-396.
24 - Zurawski, G., Bottomley, W. and Whitfeld, P.R. (1982) Proc. Nat. Acad. Sci. U.S.A. 79, 6260-6264.
25 - Di Pietro, A., Godinot, C. and Gautheron, D.C. (1983) Biochemistry 22, 785-792.
26 - Ovchinnikov, M.A., Modyanov, N.N., Grinkevich, V.A., Aldonova, O.E., Trebetskaya, O.E., Nazimov, I.V., Hundal, T. and Ernster, L. (1984) FEBS Lett. 166, 19-22.