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Characterization of epitopes of the yeast mitochondrial H^+-ATPase complex recognized by monoclonal antibodies

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Nine monoclonal antibodies which react with the β subunit of the yeast mitochondrial H^+-ATPase and three which react with a 25 kDa subunit of the enzyme complex (P25) have been characterized. Competitive binding studies indicated the presence of at least four antigenic regions on the β subunit of the enzyme complex. One antigenic region of the β subunit is recognized by two monoclonal antibodies RH 57.1 and RH 45.5 which inhibit the ATPase activity to different degrees. Antibody RH 48.6 appears to bind to a second region on the β subunit and has no effect on the ATPase activity. A third region of the β subunit is recognized by antibodies RH 51.4 and RH 72.1. RH 51.4 has no effect on the ATPase activity, whereas RH 72.1 stimulates ATPase activity. Antibody RH 32.4 which has no effect on the ATPase activity appears to bind to the fourth epitope of the β subunit. All three monoclonal anti-P25 antibodies, RH 66.3, RH 41.2 and RH 37.0, apparently bind to the same antigenic region on this subunit. Two of the monoclonal anti-β antibodies RH 48.6 and RH 51.4 were found to be very effective in immunoprecipitating the whole H^+-ATPase complex in a solid phase system. However, the other monoclonal antibodies (and also a polyclonal antiserum) appear to induce the dissociation of one or more of the H^+-ATPase subunits by their binding to the epitopes on the β or the P25 subunits.

Introduction

The mitochondrial H^+-ATPase complex (ATP phosphohydrolase, EC 3.6.1.3) has been the subject of intensive investigations in recent years. This enzyme complex is the terminal enzyme in oxidative phosphorylation and catalyzes in vivo the synthesis of ATP utilizing the proton-motive force generated by the mitochondrial electron-transport chain (see Ref. 1 for a review). In yeast, the enzyme complex has been proposed to consist of ten different protein subunits [2]. Five subunits which are imported from the cytoplasm (α, β, γ, δ and ε) [3,4] comprise the F_1 sector of the enzyme complex that contains the catalytic site for ATP synthesis and hydrolysis. The F_1 sector is physically and functionally associated with a membrane (F_0) sector which consists of three mitochondrially synthesized proteins (subunit 6, 8 and 9) and has been shown to act as a proton channel [5]. The identity of the two other polypeptides which have apparent M_r 18000 (P18) and 25000 (P25) has not been established, but they consistently co-purify with the H^+-ATPase when the enzyme complex is isolated by using a monoclonal antibody against the β subunit of the enzyme complex [6–8].

Our laboratory is currently involved in an extensive investigation into the assembly pathway of the H^+-ATPase complex, and in the elucidation of functionally important structural features of vari-

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ous subunits of the enzyme complex. As an essential part of this study, we have isolated fourteen monoclonal antibodies against the yeast mitochondrial H^+-ATPase. One of these antibodies reacted with the α-subunit of the enzyme complex (M_r 56000), nine with the β-subunit (M_r 54000), and four with the M_r 25000 polypeptide [6]. Two of these monoclonal antibodies have been central to our characterization of the H^+-ATPase assembly defects in various mutants of yeast [2,7–9], which have led us to propose an assembly pathway for the mitochondrially synthesised F_0 subunits of the enzyme complex [2,9].

In order to obtain a better understanding of the structural relationships between the various epitopes on the H^+-ATPase, we have further characterized the anti-H^+-ATPase monoclonal antibodies with respect to their ability to immunoprecipitate the H^+-ATPase complex in an intact form, their ability to compete against each other for binding to epitopes on the β or 25 kDa subunits, and their effect on H^+ATPase activity. Results of this study are presented and indicate the presence of at least four epitopes on the β subunit. Two of the antibodies which inhibit the ATPase activity recognize one of these epitopes. The other three epitopes are recognized by the monoclonal antibodies that have no effect on the ATPase activity. There is only one epitope recognized by all the monoclonal anti-P25 antibodies. Antibodies which bind to different epitopes, and have different effects on the activity of the H^+-ATPase complex will be useful for defining the various structural and functional domains of the enzyme subunits. Monoclonal antibodies against F_1-ATPase subunits from pig heart and *Escherichia coli* have been isolated in other laboratories [22–24]. Some of these antibodies might be directed against epitopes on the β subunit, which are different from that observed in yeast.

Materials and Methods

Production and purification of antibodies

The preparation of monoclonal anti-β and anti-P25 antibodies has been described previously [6]. To obtain large quantities of antibodies, cloned hybridomas were grown as ascitic tumours in pristane-primed Balb/c mice. Ascites fluid was collected 10–14 days later. Antibodies secreted by all of the hybridoma cell lines were found to be of the IgG class. Monoclonal antibodies were then purified from ascites fluid by protein A-Sepharose affinity chromatography [10]. The antibodies were labelled with ^{125}I using chloramine T [11], and the resulting specific activities ranged from $2 \cdot 10^6$ to $6 \cdot 10^6$ cpm/μg protein.

Direct binding assay of antibody affinity

The mitochondrial H^+-ATPase used as the antigen in this assay was prepared essentially as described by Tzagoloff and Meagher [3] as outlined by Murphy et al. [12]. Nitrocellulose-based wells of millititer HA plates (Millipore Corp., Massachusetts) were coated with purified H^+-ATPase (0.5 μg per well) in phosphate-buffered saline at 37°C overnight. The remaining binding sites in the wells were subsequently blocked by using 1% bovine serum albumin in phosphate-buffered saline (200 μl per well) at 37°C for 1 h. The plates were washed extensively with phosphate-buffered saline containing 1.5 mM $MgCl_2$/0.05% Tween 20/2.0 mM β-mercaptoethanol (buffer 1). Solutions of monoclonal antibodies, which contain 50000 dpm of the ^{125}I-labelled antibody and different concentrations of the unlabelled antibody (0.01–100 μg per well), were added to each well and incubated for 4 h at 37°C. After the removal of unbound antibodies by extensive washing with buffer 1, the filters at the base of the wells were punched, and the amount of bound ^{125}I-labelled antibodies was determined in a gamma counter. The amount of antibody bound to the antigen-coated filters was plotted against the concentration of antibody added in each assay. The relative affinities of the various monoclonal antibodies were compared by determining the concentration of each antibody which must be added to obtain the binding of 100 ng of antibody.

Competitive radioimmunoassay

Wells of millititer HA plates were coated with purified H^+-ATPase as above. Fixed amounts of ^{125}I-labelled antibody (50000 dpm) were incubated for 10–15 min with different concentrations of other unlabelled antibodies (0.3–100 μg per well) in a microtitre plate with its binding sites

already blocked with 1% bovine serum albumin. These mixtures were then transferred to the ATPase-coated plates and incubated for 4 h at 37°C. After extensive washing with buffer 1, the filters were punched and the radioactivity determined in a gamma counter. The amounts of labelled antibody bound in the presence of various concentrations of competing antibodies were expressed as percentage of labelled antibody bound to the antigen-coated well in the absence of any competition. The concentration of competitor required to inhibit by 50% the binding of labelled antibody was then calculated (I_{50}). In order to compare the effects of different competing antibodies on the binding of a particular labelled antibody, the I_{50} concentrations were expressed relative to the I_{50} of the same antibody that was labelled (e.g., relative to the concentration of RH 51.4 required to give 50% inhibition of ^{125}I-labelled RH 51.4; which then has a relative I_{50} value of 1). (see Fig. 2 and Table II).

Immunoprecipitation of H^+-ATPase complex

Yeast cells of wild-type strain J69-1B α ade 1 his [rho$^+$] grown in batch culture in liquid medium containing 1% w/v glucose, 1% w/v yeast extract and a salt mixture [13] were suspended at 6 mg cell dry weight/ml in low sulphate medium [14] and total cell proteins were labelled with [^{35}S] sulphate (500 μCi/ml) at 28°C for 4 h [12]. Mitochondria were then isolated essentially as described by Roberts et al. [15] and suspended in 4 mM Tris-acetate buffer (pH 7.5) containing 0.25% Triton X-100, 2 mM ATP and the protease inhibitors 10 mM *para*aminobenzamidine-HCL, 10 mM ε-amino-*N*-caproic acid and 2 mM phenylmethylsulphonylfluoride (buffer 1) [12]. After incubation at 0–4°C for 10 min, the suspension was centrifuged at 106 000 × *g* for 20 min. H^+-ATPase was then immunoprecipitated from the triton extract of the mitochondria with the monoclonal antibody(ies) or with a rabbit antiserum raised against purified H^+-ATPase. *Staphylococcus aureus* was used to precipitate antibody-antigen complexes by virtue of its surface protein A [16] essentially as described previously [17]. *Staphylococcus aureus* was mixed with polyclonal or monoclonal antibody in phosphate-buffered saline containing 0.1% sodium dodecyl sulphate (SDS)/0.5% Nonidet P40/0.1% bovine serum albumin (buffer 2). After 40 min, the antibody-absorbent complex was washed three times with buffer 2 and then buffer 1 prior to the addition of the mitochondrial triton extract. Immunoprecipitates were analysed on a 12.5% polyacrylamide gel in the presence of 0.1% SDS as described by Murphy et al. [12] and visualised by fluorography [18].

Results

Relative binding affinities of the monoclonal anti-H^+-ATPase antibodies

The relative affinities of the monoclonal antibodies were investigated by a direct binding assay using H^+-ATPase-coated solid phase and ^{125}I-labelled monoclonal anti-β antibodies RH 19.1, 28.1, 71.2, 48.6, 51.4, 32.4, 57.1, 45.5 and 72.1, and monoclonal anti-P25 antibodies RH 37.0, 66.3 and 41.2 as described in Materials and Methods.

Representative binding curves for two of these antibodies are shown in Fig. 1. The relative binding affinities of different antibodies, were de-

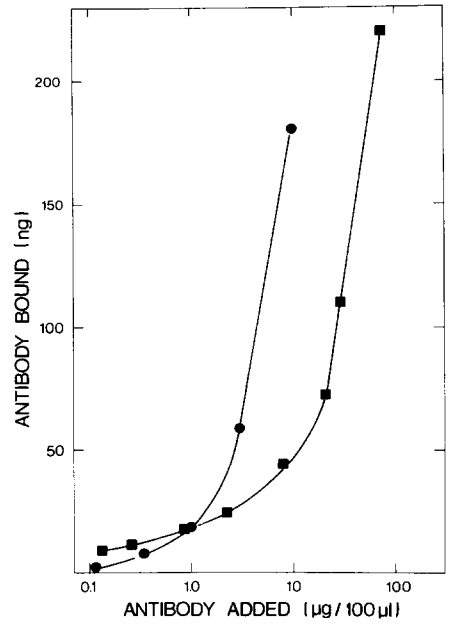

Fig. 1. The binding curves of monoclonal antibodies RH 19.1, and RH 51.4 to the H^+-ATPase complex. Various amounts of ^{125}I-labelled antibodies were incubated in H^+-ATPase-coated microtitre plates. The amount of antibody bound was determined after extensive washing then counting as described in Materials and Methods. Results presented are the average of two sets of experiments. RH 19.1 (■); RH 51.4 (●).

TABLE I

RELATIVE BINDING AFFINITIES OF MONOCLONAL ANTI-H^+-ATPASE ANTIBODIES

A direct binding assay was used to determine the relative affinity of the ^{125}I-labelled monoclonal anti-H^+-ATPase antibodies as described in Materials and Methods. The antibody concentration required to achieve a certain level of antigen binding was determined and these concentrations were expressed relative to that of monoclonal antibody RH 19.1.

Clone	Comparative binding (relative to RH 19.1)
Anti-β subunit	
RH 19.1	1
28.1	< 0.05
71.2	0.2
48.6	0.1
51.4	0.2
32.4	< 0.05
57.1	0.05
45.5	0.2
72.1	< 0.05
Anti-P25 subunit	
37.0	< 0.05
66.3	< 0.05
41.2	< 0.05

termined from such curves and expressed relative to that of RH 19.1 (= 1) which had the highest binding affinity (see Materials and Methods). Antibodies RH 71.2, 48.6, 51.4 and 45.5 were found to have a binding affinity (3–10)-fold lower than 19.1 in this solid phase binding assay (Table I). Antibodies RH 28.1, 32.4, 57.1 and 72.1 and three P25 antibodies have very low affinities, of the order of at least 20-fold lower (see Table I for a summary).

Competition between different monoclonal anti-H^+-ATPase antibodies

In order to investigate the competition between the various monoclonal antibodies for binding to their epitopes, each of the ^{125}I-labelled monoclonal antibodies RH 57.1, 45.5, 48.6, 51.4, 72.1 and 66.3 was incubated with various concentrations of unlabelled monoclonal antibodies. The other monoclonal antibodies were not used for the competitive binding experiments because only low levels of binding were observed with the iodinated antibodies.

A representative competitive binding curve between the different monoclonal antibodies and

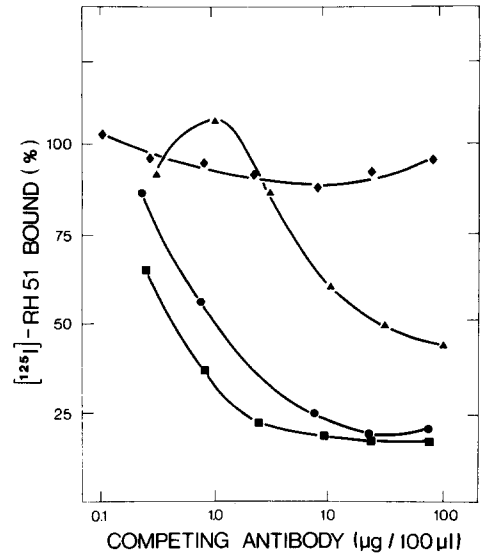

Fig. 2. Competition between ^{125}I-RH 51.4 antibody with monoclonal antibodies RH 71.2, 19.1 and 66.3. The binding of ^{125}I-RH 51.4 antibody to H^+-ATPase-coated wells was determined in the presence of various concentrations of unlabelled antibodies RH 71.2, 19.1, 51.4 and 66.3 as described in Materials and Methods. Results presented are the average of two sets of experiments. RH 51.4 (●); RH 72.1 (■); RH 19.1 (▲); RH 66.3 (◆).

RH 51.4 is shown in Fig. 2; ^{125}I-RH 51.4 was incubated with different concentrations of each of the unlabelled anti-β antibodies RH 51.4, RH 72.1, RH 19.1 and a monoclonal anti-P25 antibody RH 66.3 as a non-competing control. A progressive decrease in the binding of ^{125}I-RH 51.4 to the ATPase complex can be demonstrated with increasing concentrations of unlabelled RH 72.1, RH 19.1 or RH 51.4 itself. The amount of RH 72.1 required for 50% inhibition of the binding of labelled antibody RH 51.4 to antigen (I_{50}) was one third of the amount of unlabelled RH 51.4, whereas the amount of RH 71.2 and RH 19.1 needed were 10- and 30-times that of RH 51.4, respectively (see Table II). The other anti-β and anti-P25 antibodies RH 57.1, RH 45.5, RH 48.6, RH 32.4, RH 28.1, RH 41.2, RH 37.0 and 66.3 did not inhibit the binding of RH 51.4 even when added at 500-fold excess.

In agreement with the above results, when ^{125}I-labelled monoclonal antibody RH 72.1 was incubated with unlabelled RH 51.4, the I_{50} of RH 51.4 is 8 times that of the I_{50} of RH 72.1. Furthermore, other antibodies did not compete with the

TABLE II

COMPETITION BETWEEN DIFFERENT MONOCLONAL ANTI-H^+-ATPASE ANTIBODIES FOR BINDING TO THE H^+-ATPASE COMPLEX

Competition of monoclonal anti-β and anti-P25 antibodies for their epitopes was carried out by competitive radioimmunoassay as described in materials and Methods. The concentration of competitor required to inhibit by 50% the binding of labelled antibody was calculated (I_{50}). In order to compare the effects of different competing antibodies on the binding of a particular labelled antibody, the I_{50} concentrations were expressed relative to the I_{50} of the same antibody that was labelled. * means not competing (at least 500-times excess of the above monoclonal antibodies used for the competitive binding assay and did not show any significant inhibition).

Labelled antibodies	Relative concentration of competing antibody required for 50% inhibition											
	anti-β antibodies									anti-P25 antibodies		
	57.1	45.5	48.6	51.4	72.1	32.4	71.2	28.1	19.1	66.3	41.2	37.0
RH 51.4	*	*	*	1	0.3	*	10	*	30	*	*	*
72.1	*	*	*	8	1	*	160	*	430	*	*	*
57.1	1	6	*	*	*	*	*	*	*	*	*	*
45.5	0.2	1	*	*	*	*	*	*	*	*	*	*
48.6	*	*	1	*	*	*	*	*	*	*	*	*
66.3			*							1	1	4.3

binding of antibody RH 72.1 to its epitope, except monoclonal antibodies RH 71.2 and RH 19.1. The I_{50} of these antibodies is 160 and 430 times that of RH 72.1, respectively (Table II).

Antibody RH 57.1 inhibited the binding of ^{125}I-labelled RH 45.5 to its epitope and vice versa. The amount of monoclonal antibodies RH 45.5 required for 50% inhibition of binding of antibody RH 57.1 is 6 times that of RH 57.1. This is confirmed by the reverse experiment when iodinated RH 45.5 monoclonal antibody was used, and the amount of RH 57.1 monoclonal antibody required for 50% inhibition is about one-fifth that of RH 45.5 antibody (Table II). The analysis of competitive radioimmunoassays showed that essentially no monoclonal anti-β nor anti-P25 antibodies inhibited the binding of RH 48.6 antibody to its epitope on the β subunit.

The competition between monoclonal anti-P25 antibodies for the binding to their epitopes has also been investigated. These antibodies were found to compete for a similar antigenic region. As shown in Table II, the amount of monoclonal antibodies RH 41.2 and RH 37.0 required to inhibit the binding of RH 66.3 antibody is equal to and 4.3 times that of RH 66.3, respectively.

Immunoprecipitation of H^+-ATPase complex with polyclonal and monoclonal antibodies

Initial screening of the monoclonal antibodies indicated that these antibodies vary to a great extent in their ability to immunoprecipitate the entire H^+-ATPase complex from a triton extract of yeast mitochondria [6]. Two of the monoclonal antibodies, RH 48.6 and RH 51.4 were the only ones capable of immunoprecipitating the entire H^+-ATPase complex. As shown in Fig. 3a the precipitates obtained with these two antibodies consist of ten polypeptides considered to be the true subunits of the enzyme complex: five subunits of the F_1 sector (α, β, γ, δ, and ε), three subunits of F_0 (subunit 6, 8 and 9) which are synthesized in mitochondria and two polypeptides with molecular weight of 18000 and 25000 (P18 and P25, respectively) (Fig. 3a).

Immunoprecipitates obtained with other monoclonal anti-β antibodies RH 45.5, RH 57.1, RH 28.1 and RH 72.1 contained the α, β, γ, subunits of the H^+-ATPase (Fig. 3, lanes c–f) but the subunits of the F_0 sector, the P18 and the P25 subunits appear to be missing. It is of interest to note that a number of additional bands (M_r 45000, 43000, 16000, 13 5000, and 12500) were observed in the autoradiogram of these immunoprecipitates which were not present in those obtained with RH 48.6 and RH 51.4. The nature of these polypeptides has not been investigated, but their occurrence appears to be related to the binding of specific monoclonal antibody. The absence of the F_0 sector subunits, the P18 and the P25 in the

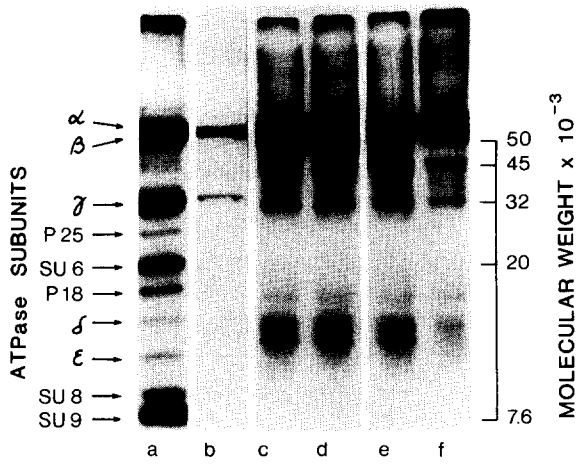

Fig. 3. Immunoprecipitation of the H^+-ATPase complex with the monoclonal anti-β antibodies. Yeast cells of wild-type strain J69-1B were labelled with [^{35}S]sulphate as described in Materials and Methods. Mitochondria were isolated from [^{35}S]sulphate-labelled yeast cells by the method of Roberts et al. [15] and a Triton extract of these mitochondria was prepared. The H^+-ATPase complex was then immunoprecipitated using monoclonal anti-β subunit antibodies in the presence of *Staphylococcus aureus* as immunabsorbent. In addition, the F_1 sector of H^+-ATPase was also immunoprecipitated from chloroform extract by monoclonal antibodies RH 48.6 or RH 51.4 (track b). The immunoprecipitated samples were displayed on a SDS-polyacrylamide gel electrophoresis and visualised by fluorography [18]. Autoradiographs of immunoprecipitates obtained by monoclonal anti-β antibodies: (a) RH 48.6 or RH 51.4; (c) RH 45.5; (d) RH 57.1; (e) RH 28.1; (f) RH 72.1.

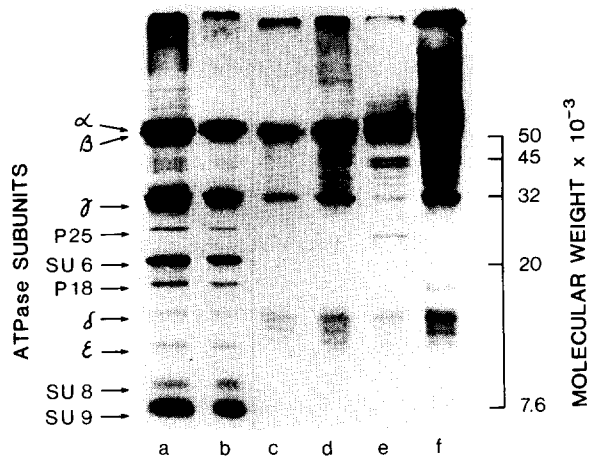

Fig. 4. Immunoprecipitation of the H^+-ATPase with the polyclonal and monoclonal antibodies. H^+-ATPase complex were precipitated from Triton extracts of ^{35}S-labelled mitochondria as in the legend to Fig. 3. The precipitates were analysed on polyacrylamide slab gels and visualised by fluorography. Shown are the immunoprecipitates obtained with (a) antibody RH 48.6; (b) antibody RH 51.4; (c) a mixture of all the monoclonal anti-β antibodies described in this paper; (d) a mixture of monoclonal antibodies as in (c) with the addition of three anti P-25 antibodies; (e) polyclonal anti-β antibody; (f) polyclonal anti-H^+-ATPase antibody.

above immunoprecipitates is presumably due to the instability of the enzyme complex, induced by the binding of some antibodies to their epitopes on the β subunit. Consistent with this observation, immunoprecipitates obtained with mixtures of monoclonal anti-β or monoclonal anti-β and anti-P25 antibodies (which also include monoclonal antibodies RH 48.6 and RH 51.4 that are capable of precipitating the entire H^+-ATPase complex) did not contain the F_0 and the P18/P25 subunits (Fig. 4c and d). Similarly, immunoprecipitates with polyclonal antibodies against the β subunit of the F_1 sector (Fig. 4e), contained the α, β, and γ subunits of the F_1 sector as well as the P25, but the other subunits were either not observed or present in greatly reduced amounts compared to the immunoprecipitate of monoclonal antibodies RH 48.6 and RH 51.4 (Fig. 4a and b). In the case of the immunoprecipitate with polyclonal anti-holo-H^+-ATPase antibody, the five F_1 subunits could be observed, but the three F_0 subunits were present in reduced amounts, while the P25, and the P18 were not visible in the autoradiogram (Fig. 4f).

Discussion

In this paper we present the results of competitive binding studies between different monoclonal antibodies to the yeast H^+-ATPase complex. The inhibition of the antigen binding of one antibody by another can be the result of: (a) the recognization of the same antigen structure by the two antibodies; (b) recognization by the two antibodies of overlapping structures which share some, but not all, of an epitope; (c) the proximity of the two distinct antigenic determinants in the folded antigen molecule which creates steric hindrance to the binding of two antibodies at one time. Thus, antibodies which compete for binding can be said to recognize the same general region of the antigen.

Analysis of competitive inhibition experiments showed that there are at least four such antigenic regions recognized by the monoclonal anti-β antibodies. One of these regions is recognized by monoclonal antibodies RH 51.4, RH 72.1, RH 71.2 and RH 19.1 (see below for a discussion), and the second region is recognized by monoclonal antibodies RH 57.1 and RH 45.5. The third region is recognized by monoclonal antibody RH 48.6 which is one of the only two antibodies capable of immunoprecipitating the whole H^+-ATPase complex in a solid phase system. Two other monoclonal antibodies to the β subunit, RH 32.4 and RH 28.1, can not be assigned to the above antigenic regions. Since they did not inhibit the binding of the other anti-β antibodies, they could recognize one (or two) other epitopes.

It is interesting to note the relationship between the antigenic region to which the anti-β antibodies bound, the effects of the antibodies on the H^+-ATPase activity, and the ability of the monoclonal anti-β antibodies to immunoprecipitate the entire H^+-ATPase complex (see Table III). Firstly, antibodies RH 57.1 and RH 45.5 which recognized a similar antigenic region of the β molecule were both found to inhibit the enzyme activity. This may indicate that these antibodies bind to the ATPase catalytic site on the β subunit, or that the binding of these antibodies to their epitope elsewhere induces conformational changes which indirectly affect the catalytic site on the β subunit. The extent of inhibition of the enzyme activity by these antibodies, however, is not the same (and is inversely proportional to their binding affinity to the β-subunit) indicating that they do not recognize identical epitopes. Preliminary kinetic studies of the ATPase activity indicates that monoclonal antibody RH 57.1 inhibits the H^+-ATPase complex in a non-competitive manner. Thus, this antibody does not appear to be directed against the catalytic site on the β subunit.

TABLE III

SUMMARY OF THE PROPERTIES OF THE ANTI-β SUBUNIT MONOCLONAL ANTIBODIES WITH RESPECT TO THEIR EPITOPES, EFFECTS ON THE ATPASE ACTIVITY AND ABILITY TO IMMUNOPRECIPITATE INTACT H^+-ATPASE COMPLEX

An asterisk indicates significant changes in ATPase activity.

Epitopes on the β subunit	Clone	Inhibition of ATPase activity (% inhibition by 100 μg IgG)	Ability to immuno-precipitate intact H^+-ATPase complex
I	RH 72.1	−25 *	−
	51.4	−6	+
	71.2	8	−
	19.1	4	−
II	57.1	90 *	−
	45.5	36 *	−
III	48.6	7	+
IV	32.4	−3	−
V(?)	28.1	2	−

Another antigenic region is recognized by antibody RH 51.4 which has no effect on the ATPase activity, and by RH 72.1 which stimulates the ATPase activity. The binding affinity of RH 72.1 is at least (4–8)-fold lower than RH 51.4, and the binding of both antibodies is partially inhibited by monoclonal antibodies RH 19.1 and RH 71.2 (Tables I and II). In addition, antibody RH 51.4 is capable of precipitating the entire H^+-ATPase complex, whereas the RH 72.1 immunoprecipitate contains only the α, β, and γ subunits of the H^+-ATPase. Thus, RH 72.1 and RH 51.4 do not bind identical epitopes, but may bind to adjacent or overlapping epitopes.

Only two of the above monoclonal antibodies could immunoprecipitate the entire enzyme complex. Interestingly, these antibodies appear to be directed against different antigenic regions. Observations from several laboratories indicated that immunoprecipitates of polyclonal antibodies raised against one or more subunit(s) of the H^+-ATPase complex often do not contain some of the H^+-ATPase subunits [19–21]. One possible explanation is that the binding of antibodies to some antigenic determinants on the H^+-ATPase complex induces conformational changes resulting in the dissociation of some subunits from the enzyme complex. We observed this phenomenon by using mixtures of monoclonal anti-β or monoclonal anti-β and anti-P-25 antibodies. It is therefore essential to use an appropriate monoclonal antibody in the immunoprecipitation studies of H^+-ATPase assembly. The above observations might have more general implications of the necessity to

use monoclonal antibodies in the study of other multi-subunit enzyme complexes.

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