IMMUNOCHEMICAL EVIDENCE FOR STRUCTURAL HOMOLOGIES BETWEEN MAMMALIAN CARDIAC AND SKELETAL MYOSINS

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1. Introduction

Few cross reactions have been observed between highly purified myosins extracted from the cardiac, skeletal and smooth muscles of the same animal [1–4]. This led to the conclusion that the antigenic structure of muscular myosin is tissue specific. However it is puzzling to postulate from these data that cardiac and skeletal myosins have no common structures. These two molecules have close physicochemical and biological properties, and homologies exist between the few peptides which have been analysed [5–8]. The conclusion that myosin was tissue specific was derived from studies carried out with antibodies elicited either by the native molecule [1,3,4] or by its major subunits, the heavy chains, removed under dissociating conditions [2]. One explanation of the low cross reactivity observed between the myosins extracted from different muscle types could be that the common sites, if they exist, are not reactive in the above immune systems.

Immunogenic sites not represented in a native molecule can be exposed by various approaches. Enzymatic cleavage has been used for myosin, and new determinants became reactive with antisera elicited by the sub-fragment S1, obtained by papain cleavage [9] or by heavy meromyosin, HMM, prepared by tryptic hydrolysis [10].

In the present study, we reinvestigated the muscle-type specificity of myosin with antibodies induced by heavy meromyosins. As shown [10,11], such antibodies, if elicited by purified HMM, are highly specific to HMM and the corresponding intact myosin. The antigenic structures of cardiac and skeletal myosins were compared in two species, the rabbit and the rat, with guinea-pig antisera to rabbit skeletal, rabbit cardiac and rat cardiac HMM. From cross reactions observed by quantitative microcomplement fixation, MCF [12] we present evidence that myosins extracted from the cardiac and skeletal muscles of the same animal species contain common epitopes. This indicates the existence of strong structural homologies in these proteins.

2. Experimental

Myosins were prepared from the back and legs or the cardiac ventricles of New Zealand rabbits and Wistar rats, by minor modifications of the procedure in [11,13]. Crude extracts refers to the material extracted in the Guba-Straub solution, and dialysed against 10 mM Tris–HCl, 0.3 M KCl, pH 7.6. Heavy meromyosins were obtained by tryptic digestion of the myosins at 27°C in a solution containing 0.1 M Tris–HCl, 0.6 M KCl, 0.1 mM $CaCl_2$, pH 7.7, with trypsin/myosin ratio 1 : 250 (w/w). The purification of HMM by chromatography on Sepharose 6B and the preparation of antisera to purified HMM were carried out as in [10]. Five guinea-pigs were immunized with each type of HMM, and antiserum pools were obtained by combining in equal proportions the five individual sera of a particular bleeding. Quantitative micro-complement fixation was performed according to [12], in final vol. 0.7 ml.

3. Results

Figure 1 presents the complement fixation reactions of rabbit cardiac and skeletal myosins or crude extracts with antibodies to pure rabbit skeletal HMM (left) and rabbit cardiac HMM (right). Both antisera led to comparable effects:

(i) The curves obtained with the homologous and the heterologous antigens were very close.

(ii) The extent of cross reactions was identical with the myosins and the tissular extracts.

These data indicate common antigenic determinants between the two types of myosins. The similar responses given by the myosins and the crude extracts confirm our observation [11] that in the HMM–antiHMM immune system, the degree of purity of myosin does not affect its antigenic activity.

Cross reactivity measurements between proteins can be affected by various parameters [14], such as the length of the immunization program, the degree to which the results of reciprocal test agree and the variability of the antibody producers. The effect of these factors was studied, and the assays were performed only with the crude extracts to simplify the procedure. Figure 2 presents data on fixation of rabbit cardiac and skeletal tissular extracts reacting

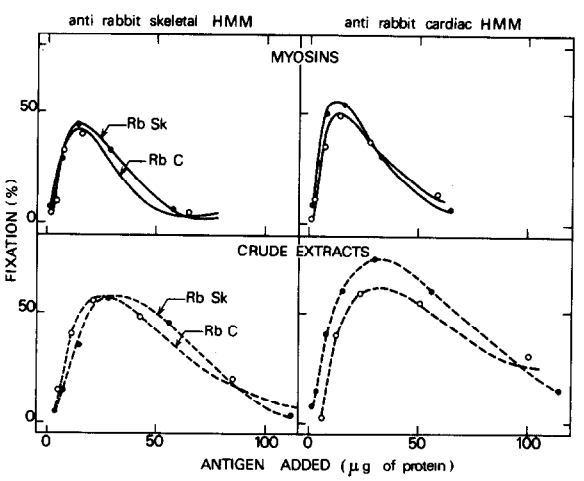

Fig.1. Microcomplement fixations with anti-rabbit white skeletal HMM antiserum Pool 18-4, diluted 1 : 400 (left) and anti-rabbit cardiac HMM antiserum Pool 29-4, diluted 1 : 220 (right). (•——•) RbSk, rabbit white skeletal myosin; (o——o) Rbc, rabbit cardiac myosin; (•– – –•) rabbit white skeletal crude extract; (o– – –o) rabbit cardiac crude extract.

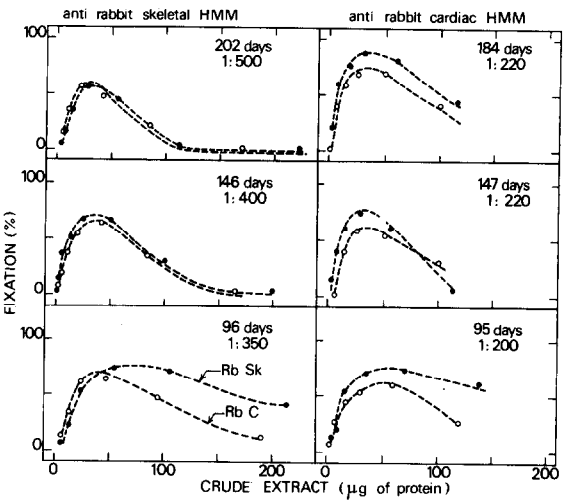

Fig.2. Microcomplement fixations with antisera against rabbit white skeletal HMM (left) and rabbit cardiac HMM (right), reacting with crude extracts of rabbit white skeletal ((•– – –•) RbSk) and cardiac ((o– – –o) Rbc) muscles. For each antiserum pool, the time after initial immunization and the dilution used in the assay are indicated.

with antisera withdrawn 3, 4 and 5 months after initial immunization. With both types of antisera, the reciprocal recognitions of cardiac and skeletal myosins were apparent throughout the immunization program, showing that in this respect, antiserum specificity did not vary with time. After 3 months of immunization, the shape of the curves became sharper too in both cases. This evolution, which can be interpreted as a consequence of the maturation of the immune response, was the same, for each type of antiserum, with the cardiac and skeletal antigens, indicating that the two proteins behaved identically from this point of view too. The degree of cross reaction was not absolutely reciprocal: after 3 months, antiskeletal HMM sera did not differentiate heart and muscle (fig.2, left), whereas, with anticardiac HMM sera (fig.2, right), the heights of the curves were around 15% higher with the muscle than with the heart. This difference, though hardly significant, was reproducible from one experiment to the other, indicating an unperfect agreement of the reciprocal tests. Deviations of reciprocity have already been observed in immunochemical studies of other proteins [14]. Individual guinea-pig variability was tested

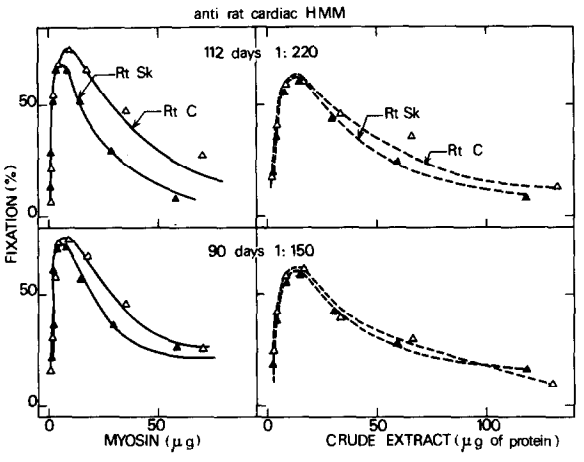

Fig.3. Microcomplement fixations with antisera induced by rat cardiac HMM, and with drawn 90 days (bottom) and 112 days (top) after initial immunization. (▲——▲) RtSk rat skeletal myosin; (△——△) RtC rat cardiac myosin ; (▲– – –▲) rat skeletal crude extract; (△– – –△) rat cardiac crude extract.

with the anticardiac HMM sera of the fourth bleeding (147 days). The heterologous skeletal antigen was recognized by each individual antiserum (not shown), indicating that each animal produced cross-reacting antibodies and that the measurements made with the pool reflected each individual response.

Data on the comparison of cardiac and skeletal myosins in the rat are shown in fig.3. Rat skeletal myosin strongly cross reacted with the antirat-cardiac HMM antibodies. As for the rabbit, the reactions of rat cardiac and skeletal myosins were almost identical, and this close relationship was observed with the crude extracts as well as with the myosins. For the rat also, length of the immunization program did not interfere, since the extent of cross-reaction did not vary between 3 and 4 months after initial immunization.

4. Discussion

The results reported here clearly show that in the rabbit and the rat, cardiac and skeletal myosins contain common antigenic reactive regions. The hypothesis that these sites are traces of a highly immunogenic impurity linked to the myosins seems improbable. Various observations [11] have shown that antibodies to purified HMM are specific to HMM and myosin, and that the other contractile proteins (tropomyosin, troponin, protein C) do not interfere in the MCF reactions. Moreover, the absolute degree of reciprocal recognition observed in the rabbit throughout the immunization program (fig.2) implies that the putative contaminant has the same structure in the skeletal muscle and the heart, and that it is highly immunogenic. It is very unlikely that these conditions could be simultaneously fulfilled.

It can therefore be reasonably postulated that the cross reactions we observed between the cardiac and skeletal myosins of the same animal species were due to structures contained within the two molecules themselves and thus, that these two proteins have common epitopes located on HMM. This conclusion is in full agreement with the data obtained by immunofluorescence with antimyosin autoantibodies detected in the sera of two patients [15]. Both antisera recognized on myosin only the HMM fragment, and stained equally the myofibrils of skeletal and cardiac tissues. One of them even stained smooth muscle and cytoplasmic myosin. The existence of common epitopes between cardiac and skeletal myosins of the same animal was previously suggested by some observations made with antibodies against intact myosins [3,4], but they could hardly be detected. It is conceivable that the common epitopes are more immunogenic when HMM is the immunogen, probably, as was first hypothetized, because they are more favorably exposed in that case.

The skeletal muscles used in the present report were not homogeneous products. Muscles of the back and legs of the rabbit contain mainly white fibers, but rat muscles are composed of white, intermediate and red fibers [16]. Information on the structure of the myosins extracted from these three types of fibers is limited, but the available evidence indicates several differences (reviewed [17]). It can be hypothetized that several isoenzymes of myosin are present within the so-called 'skeletal myosin'. In spite of this heterogeneity, the extent of cross reaction between the skeletal muscle and the heart was similar in the rabbit and the rat (fig.1,2). This suggests that the epitopes common to cardiac and skeletal myosins are present on the various types of skeletal fibers and therefore that the myosins of these fibers share antigenic determinants. A similar conclusion was recently

drawn from studies carried out on the rat diaphragm with an immunocytochemical approach [18].

The antigenic structures of cardiac myosins extracted from various animal species are different, when compared with antibodies induced by heavy meromyosins [11]. For the sites involved in these immune systems, cardiac and skeletal myosins of the same species are more closely related than cardiac myosins of two different species. In other respects, it is not surprising that rabbit skeletal and pig cardiac myosins do not cross react, though this observation was otherwise interpreted [10].

The immunological relationship between the myosins from different muscles of the same animal species indicate an underlying structural similarity for the sites concerned. This finding is consistent with the limited information obtained by other approaches. Amino acid sequence and peptide mapping studies show differences, but also similarities in the cat [19], the rabbit [20] and the chicken [21]. It is obvious that much additional work is required to determine the structure and localisation of the antigenic determinants of myosin. Nevertheless, the immune investigation of the molecular features of this protein with antiHMM antibodies offers a new approach for comparative studies.

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