Investigation of Immunological Relationships among Myosin Light Chains and Troponin C†

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ABSTRACT: Two classes of myosin light chains can be distinguished functionally: those that restore calcium regulation to "desensitized" scallop myofibrils, and those that do not (Kendrick-Jones, J., et al. (1976), *J. Mol. Biol. 104,* 747–775). Despite this functional classification, chemical analyses reveal few patterns unique to regulatory light chains, and, indeed, sequence comparisons suggest structural similarities between both classes of myosin subunits (Collins, J. H. (1977), *Nature (London) 259,* 699–700; Kendrick-Jones, J., and Jakes, R. (1977), in International Symposium on Myocardial Failure at Tegernsee, Riecker, G., and Boehringer, Ed., Munich, West Germany, Springer-Verlag, pp. 28–40). Immunological assays using antisera to regulatory and to nonregulatory light chains showed no correlation between antigenic activity and the presence or absence of regulatory function. Weak cross-reactivity was observed, however, among myosin light chains and troponin C, consistent with the suggestion made on the basis of sequence homologies that these subunits contain similar structural domains (Weeds, A. G., and McLachlan, A. D. (1974), *Nature (London) 252,* 646–649). Unexpectedly, the strongest cross-reactivity observed was that between the vertebrate myosin alkali 1 and DTNB light chains.

The general structure of the myosin molecule appears to have been conserved throughout the phyla. Myosins isolated from a variety of vertebrate and invertebrate species have a similar subunit composition: two chains with a molecular weight of about 200 000 and four smaller subunits with molecular weights between 16 000 and 22 000 (Lowey and Risby, 1971; Burridge and Bray, 1975; Kendrick-Jones et al., 1976). Electron micrographs of all myosin molecules so far examined show a tail of about 1500 Å attached to two globular heads (Elliot et al., 1976). Enzymatic digestion of myosins invariably produces soluble subfragments with ATPase activity, and water-insoluble α-helical rods which are responsible for the aggregation properties of myosin. The similarity in overall morphology and charge distribution of many myosins is also indicated by their assembly into filaments which contain the same axial periodicity (for review, see Squire, 1975).

An important difference among species is in their mode of calcium regulation of muscle contraction. In vertebrate muscles, calcium activates ATPase activity by binding to the troponin complex on the thin filament (Ebashi et al., 1969); whereas, in molluscan muscles, calcium increases ATPase activity by binding to myosin (Kendrick-Jones et al., 1970). Removal of a particular light chain from scallop myosin with EDTA[1] (the "EDTA light chain") results in the loss of calcium sensitivity (Szent-Györgyi et al., 1973; Kendrick-Jones et al., 1976). The ATPase of such a "desensitized" myosin preparation is fully active regardless of the calcium concentration. Calcium regulation is restored upon recombination of this light chain with myosin. Surprisingly, one of the vertebrate myosin light chains, the "DTNB light chain," can also restore calcium sensitivity to desensitized scallop myosin, despite the absence of myosin-linked regulation in vertebrate striated muscles (Kendrick-Jones, 1974).

In fact, a large class of myosin light chains, including those from lobster tail, mussel adductor, bovine heart, chicken gizzard, and frog leg muscles, shares the ability to substitute for the native regulatory light chain of scallop myosin as a mediator of calcium sensitivity (Kendrick-Jones et al., 1976). By this criterion, one can distinguish two classes of light chains within each species of myosin: those that restore calcium sensitivity, the so-called "regulatory light chains," and those that do not.

While enzymatic studies have led to such a classification of light chains, peptide maps and amino acid analyses have revealed few patterns unique to regulatory light chains (Kendrick-Jones et al., 1976). Instead, sequence comparisons have led to the conclusion that both classes of myosin light chains are homologous, not only with each other, but also with the calcium-binding proteins, troponin C and parvalbumin (Weeds and McLachlan, 1974; Collins et al., 1973; Collins, 1976; Kendrick-Jones and Jakes, 1976). However, the different functions of these proteins, as well as structural studies indicating a more extended shape for the regulatory light chains (Stafford and Szent-Györgyi, 1976) than for parvalbumin and troponin C (Kretsinger and Barry, 1975), suggest that their three-dimensional structures may not be as similar as the sequence homologies imply.

In order to analyze further the structural relationships among these proteins, we have investigated their serological cross-reactivity. In particular, we asked whether regulatory and nonregulatory light chains could be distinguished immunologically. Antisera to two regulatory light chains, that of lobster tail muscle ("LB1") and that of chicken pectoralis muscle ("DTNB l.c."), were used, as well as antiserum to the nonregulatory chicken alkali 1. The results show limited cross-reactivity among regulatory light chains and between myosin light chains and troponin C. The strongest cross-reactivity observed was that between vertebrate alkali 1 and DTNB light chains. Differences in antibody binding activity could not, therefore, be correlated with the presence or absence of regulatory function. The immunological data are, nevertheless, consistent with the suggestion made on the basis of

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[1] Abbreviations used are: EDTA, ethylenediaminetetraacetic acid; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); Gdn·HCl, guanidine hydrochloride.

sequence homology that all of these subunits contain similar structural domains.

Materials and Methods

Proteins. Myosin was prepared from chicken breast muscle as described by Holtzer and Lowey (1959). The preparation of purified light chains was carried out as described earlier (Holt and Lowey, 1975).

Rabbit immunoglobulin was precipitated from serum by 40% saturated ammonium sulfate (4 °C) containing 0.4 mM EDTA and dialyzed against 0.0175 M potassium phosphate, pH 6.3. The protein was lyophilized and stored at 4 °C.

Rabbit myosin DTNB l.c., scallop myosin EDTA l.c., lobster myosin LB1, and rabbit troponin C (TnC) were gifts of S. S. Margossian, A. G. Szent-Györgyi, J. Regenstein, and K. Y. Lee, respectively.

Sodium Dodecyl Sulfate Gel Electrophoresis. Electrophoresis on 10% polyacrylamide gels containing 0.1% sodium dodecyl sulfate and 0.1 M sodium phosphate (pH 7) was carried out as described by Weber and Osborn (1969). Gels were fixed in 45% methanol, 9% acetic acid overnight and stained for 1 h with 0.25% Coomassie brilliant blue in the same solvent. Gels were destained by diffusion in 7.5% acetic acid and 5% methanol. Iodinated antigens were coelectrophoresed with unlabeled protein. After destaining, gels were frozen through contact with dry ice and sliced with a Mickle gel slicer. Slices were counted in a well-type γ counter.

Iodination. Antigens were labeled with ^{125}I using Chloramine-T according to Hunter (1970). Initially, the Chloramine-T:protein ratio was varied, with the incorporation of iodine into protein increasing with added Chloramine-T. Antibody binding activity, as assayed by the method of Hunter (1970), did not vary with the Chloramine-T:protein ratio. Thereafter, the amount of Chloramine-T used (21% of protein, w/w) was sufficient to give 50–75% incorporation of iodine. Typically, 20 μCi of ^{125}I in 0.1 N NaOH (New England Nuclear), together with carrier potassium iodide (equimolar to protein), was neutralized in phosphate buffer, pH 7.5, 0 °C. Chloramine-T was added, followed immediately by 100–200 μg of protein in a total volume of 100–200 μL. The final phosphate buffer concentration was 50 mM. After stirring on ice for 15 min, the reaction was terminated by the addition of sodium metabisulfite (weight equal to that of Chloramine-T), and the mixture was brought to 0.4 mL by the addition of bovine serum albumin, 2 mg/mL, in borate-saline buffer (16 mM borate, 0.15 M sodium chloride, pH 8.2). Gel filtration (Sephadex G-50) and/or dialysis separated unreacted iodine from labeled antigen, as indicated by precipitation of greater than 96% of the radioactivity by 10% trichloroacetic acid. Dodecyl sulfate gel electrophoresis showed that each iodinated antigen migrated as a single peak of radioactivity coincident with Coomassie blue stained protein.

Preparation of Antisera. The preparation of antisera was carried out as described previously (Holt and Lowey, 1975). For this study, pools were made of bleeds collected 6 months or more after the initial immunization, since it has been shown that a broadening of antibody specificities occurs with time, and more cross-reactivity may be observed (Prager and Wilson, 1971). Separate pools were made from bleeds of two rabbits, each immunized with the same antigen. Antisera to chicken alkali 1 and DTNB l.c., antisera to lobster LB1, and antiserum to glycogen debranching enzyme were contributed by J. C. Holt, J. Regenstein, and J. Trinick, respectively. Goat serum specific for the Fc fragment of rabbit IgG was a generous gift of A. Nisonoff.

Double Diffusion. Gel diffusion was carried out with 1% agar (purified Difco) dissolved in 0.4 M KCl, 10 mM potassium phosphate, pH 7.4, as previously described (Holt and Lowey, 1975). After development of precipitin lines for 3 to 6 days at 4 °C the plates were photographed in dark field illumination.

Antibody Purification. Specific antibody was isolated from immune sera by adsorption to purified antigen coupled to Sepharose 4B (Omenn et al., 1970). Typically, 50 mL of clarified serum was applied at 40 mL/h to a column (25 × 75 mm) of Sepharose 4B containing 2 mg of coupled antigen/mL of packed gel, equilibrated in borate-saline buffer at either room temperature or 4 °C. The column was washed at 80 mL/h until the optical density at 280 nm returned to the initial baseline value. Antibody was eluted with 4 M Gdn·HCl dissolved in column buffer and dialyzed immediately against 3 × 6 L of buffer, or until Gdn·HCl was no longer detected in the dialysate upon addition of Nessler's reagent. The protein was freeze-dried and stored at 4 °C.

Radioimmunoassay. The procedure adopted was essentially as described by Kuettner et al. (1972). Antisera were assayed for antigen binding activity by incubating increasing amounts of either immunoglobulin or serum with a fixed amount (2.5–10 ng) of ^{125}I-labeled antigen (25 °C, 1 h). Non-immune IgG or serum was also present, so that total rabbit IgG was constant and sufficient to be completely precipitated upon subsequent addition of goat anti-rabbit Fc serum. After standing overnight at 4 °C, precipitates were washed and dissolved in dilute NaOH before counting in a well-type γ counter. The percent antigen bound was calculated from the radioactivity in the precipitate after subtracting the amount present in a control of non-immune precipitate. The amount of labeled antigen precipitated by non-immune rabbit IgG amounted to no more than 2–10% of the antigen added.

In competition experiments, unlabeled protein was first incubated with an amount of antibody known to be in a linear range of binding activity with respect to labeled antigen. After incubation (1 h, 25 °C), radiolabeled antigen, followed by goat antiserum, was added as before. The amount of radioactivity precipitated in the absence of inhibitor was normalized to 100%. The labeled antigen bound in the presence of inhibitor was then corrected by the same factor.

Unless otherwise stated, borate-saline buffer was used throughout.

Results

The reaction between various muscle proteins and antisera specific for light chains was initially investigated by double diffusion in agar. Antiserum to lobster LB1 forms a single precipitin line when diffused against a mixture of both lobster light chains, LB1 and LB2; serum prepared against both light chains forms two distinct precipitin lines (Figure 1a).

Antiserum to chicken DTNB l.c., when diffused against either the homologous antigen or chicken alkali 1, reacts only with immunogen (Figure 1b). Similar results were obtained with antiserum against alkali l.c. (not shown; see also Holt and Lowey, 1975). However, in the presence of high concentrations of the immunoglobulin fraction, or of purified antibody from antiserum against DTNB l.c., a faint precipitin line was formed with alkali 1 (Figure 1c and d). That the reaction is not due to a contaminant will be shown subsequently. No cross-reactions were observed in double diffusion with rabbit troponin C or scallop EDTA l.c., even with high concentrations of antibody specific for DNTB l.c.

The lack of a precipitin line does not exclude the possibility that nonprecipitating antibody–antigen complexes are formed. Precipitation of immune complexes by goat anti-rabbit im-

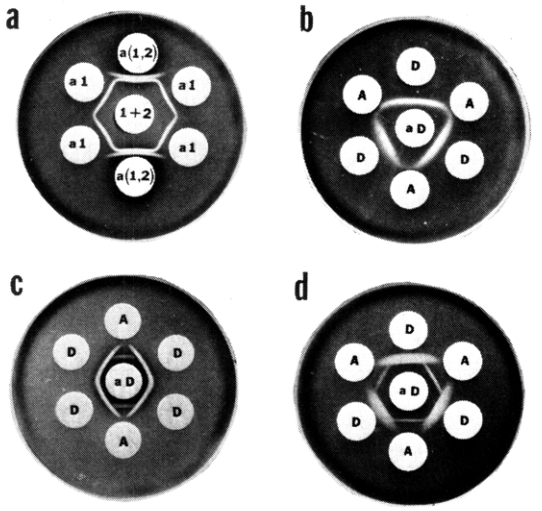

FIGURE 1: Immunodiffusion of light chains. (a) A mixture of both lobster myosin light chains is in the center well, 0.5 mg/mL. "a1" is serum prepared against LB1; "a(1,2)" is serum prepared against both LB1 and LB2. (b) Anti-DTNB l.c. serum (959) in center well; alternating clockwise from top are serial dilutions of chicken DTNB l.c. and alkali 1, respectively, from 0.1 mg/mL. (c) Anti-DTNB l.c. immunoglobulin (serum 959) in center well, 160 mg/mL; "A" is chicken alkali 1, 0.2 mg/mL; "D" is chicken DTNB l.c., 0.7 mg/mL. (d) DTNB l.c.–Sepharose purified antibody (serum 959) in center well, 24 mg/mL; alternating clockwise from top are serial dilutions of chicken DTNB l.c. and alkali 1, respectively, from 0.1 mg/mL.

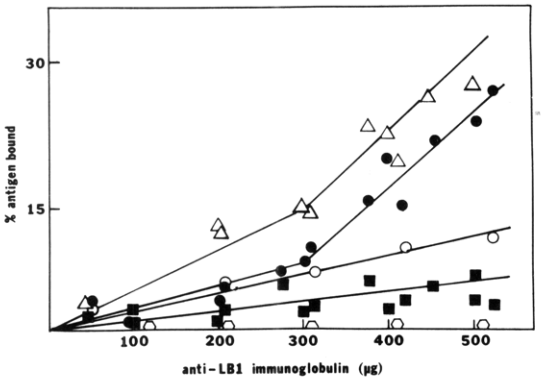

FIGURE 2: Radioimmunoassays with anti-LB1 immunoglobulin: (△) scallop EDTA l.c.; (●) chicken DTNB l.c.; (○) chicken alkali 1; (■) rabbit troponin C. (○) Reaction of anti-glycogen debranching enzyme with the same antigens. ^{125}I-labeled antigen (2.5 ng) was used in each assay.

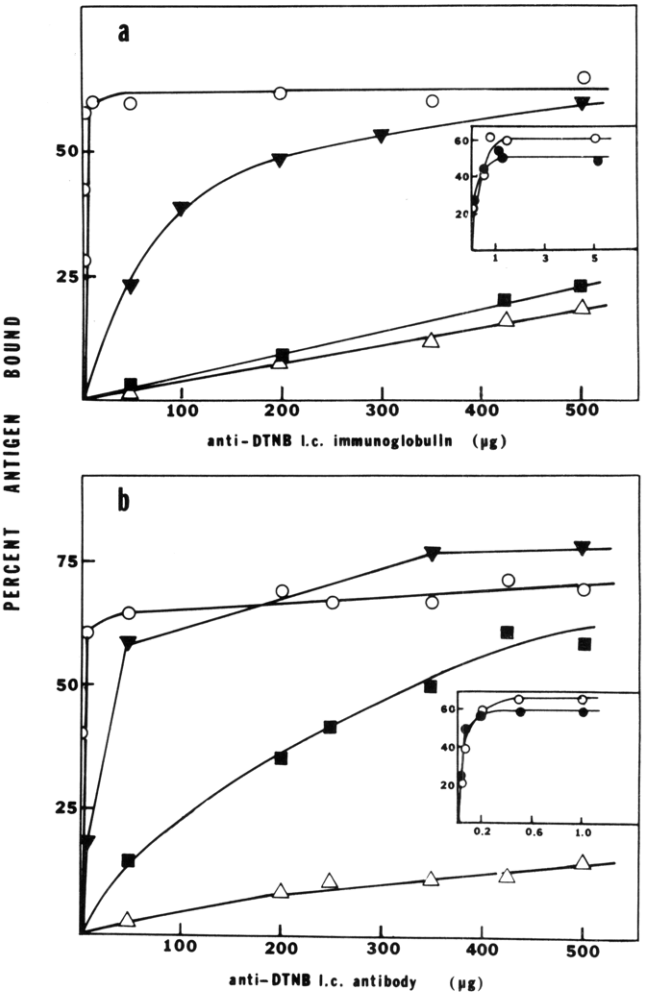

FIGURE 3: Radioimmunoassays with anti-DTNB l.c. (a) Immunoglobulin (serum 959) and (b) DTNB l.c.–Sepharose purified antibody (serum 959). (○) Chicken alkali 1; (▼) rabbit DTNB l.c.; (■) rabbit troponin C; (△) scallop EDTA l.c. Insets: (a) immunoglobulin (serum 1–18) and (b) DTNB l.c.–Sepharose purified antibody (serum 1–18). (○) Chicken DTNB l.c.; (●) chicken alkali 1. ^{125}I-labeled antigen (2.5 ng) was used in each assay.

munoglobulin allows detection of both monovalent antigens bound to antibody and low affinity interactions of antibody and antigen that form soluble complexes. Figure 2 shows the binding of scallop EDTA l.c., chicken DTNB l.c. and alkali 1, and rabbit troponin C by an immunoglobulin specific for lobster LB1. A different anti-LB1 serum contained similar amounts of total cross-reactive antibody; however, greater reactivity was observed with alkali 1 and troponin C than with either EDTA l.c. or DTNB l.c. In both cases, the cross-reactivity observed was relatively weak: homologous antigen was bound by about a 500-fold lower concentration of antibody than was necessary to bind heterologous antigen. Moreover, attempts to compete the homologous reaction (anti-LB1 + LB1) with heterologous antigens (e.g., EDTA l.c.) were unsuccessful. However, the binding of heterologous antigens to anti-LB1 was significantly greater than that obtained with immunoglobulin prepared from either a non-immune serum or from a serum specific for an unrelated protein, glycogen debranching enzyme from chicken muscle (Figure 2).

Antibodies to chicken DTNB l.c. also cross-react with scallop EDTA l.c., chicken alkali 1, and rabbit troponin C subunit (Figure 3). Consistent with the weak reactivity of [^{125}I]EDTA l.c. toward antiserum elicited by immunization with DTNB l.c., the scallop light chain is not effective as a competitor of the homologous antibody–antigen reaction (Figure 4). The most extensive cross-reactivity observed was that between DTNB l.c. and alkali 1 in the presence of antibody prepared against DTNB l.c. The possibility exists that this reaction is an artifact resulting from immunization with DTNB l.c. contaminated with traces of alkali 1. If so, purification of antibody by adsorption to Sepharose-coupled DTNB l.c. should result in a decreased reactivity toward alkali 1. The protein used in preparing the immunoadsorbent showed no detectable alkali 1, as evidenced by sodium dodecyl sulfate gel electrophoresis (Figure 5). Yet, as Figure 3b indicates, the relative reactivities of alkali 1 and DTNB l.c. did not change after antibody purification. This figure also shows that DTNB l.c. and troponin C isolated from rabbit muscle react with rabbit antiserum. Such a reaction against "self" protein in the case of antiserum against DTNB l.c. is consistent with previous observations (Holt and Lowey, 1975). Similar results were

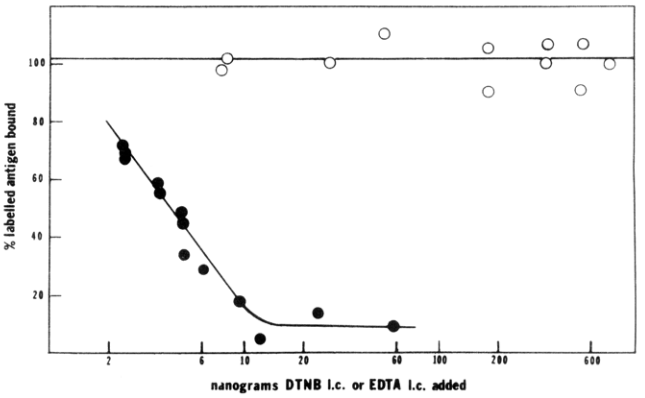

FIGURE 4: Competition of reaction of anti-DTNB l.c. immunoglobulin (serum 959) + ^{125}I-labeled chicken DTNB l.c. with chicken DTNB l.c. (●) and scallop EDTA l.c. (○). Ten nanograms of ^{125}I-labeled antigen and 0.4 μg of immunoglobulin were used.

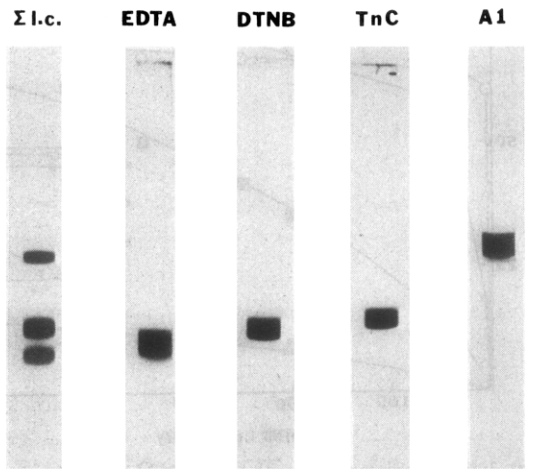

FIGURE 5: Electrophoresis of light chains on 10% acrylamide dodecyl sulfate gels. "Σ l.c." is total light chains from chicken myosin (25 μg); "EDTA" is scallop regulatory light chain (16 μg); "DTNB" and "A1" are chicken myosin DTNB l.c. and alkali 1, respectively (10 μg); "TnC" is rabbit troponin C (10 μg).

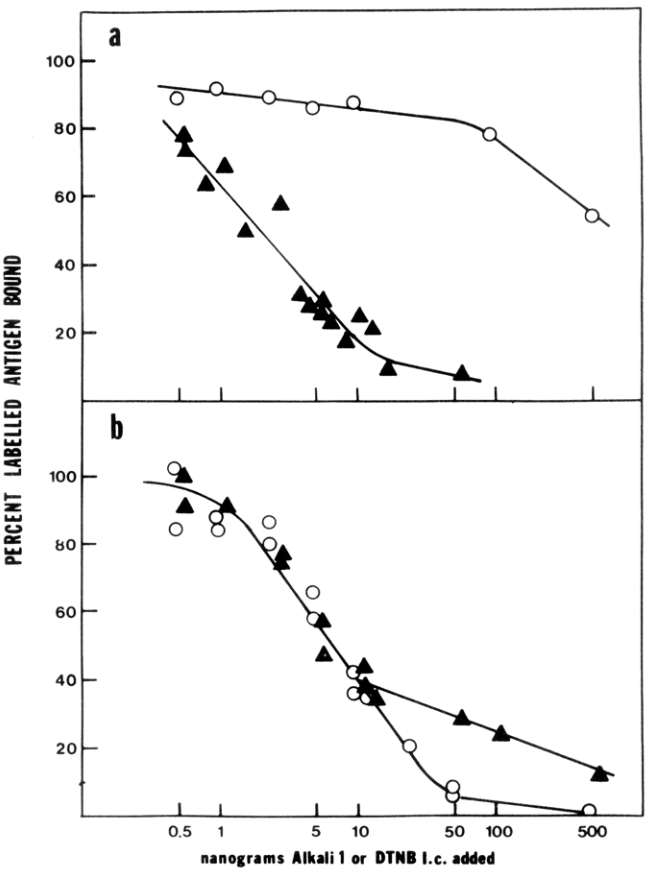

FIGURE 6: (a) Competition of anti-DTNB l.c. immunoglobulin (serum 1-18) + ^{125}I-labeled chicken DTNB l.c. by chicken alkali 1 (○) and DTNB l.c. (▲). (b) Competition of anti-DTNB l.c. immunoglobulin (serum 1-18) + ^{125}I-labeled chicken alkali 1 by chicken alkali 1 (○) and DTNB l.c. (▲). ^{125}I-labeled antigen (2.5 ng) and 0.5 μg of anti-DTNB immunoglobulin were used in both assays.

obtained using another serum prepared against DTNB l.c. (data not shown). Again, purification of the antibody by adsorption to DTNB l.c.-Sepharose did not decrease its reactivity toward alkali 1.

In view of the extensive cross-reactivity observed between anti-DTNB l.c. and alkali 1, it was expected that preincubation of anti-DTNB l.c. with alkali 1 would inhibit the subsequent reaction with homologous antigen, [^{125}I]DTNB l.c. However, alkali 1 was found to be a poor competitor of the homologous reaction (Figure 6a). That both antigens were indeed reacting with the same antibodies could be shown, however, by competition of the heterologous reaction, i.e., that of anti-DTNB l.c. immunoglobulin with [^{125}I]alkali 1, by both DTNB l.c. and alkali 1 (Figure 6b). Similar results were observed with anti-DTNB l.c. antibody isolated from an alkali 1–Sepharose affinity column (see below). Preincubation of this antibody with either DTNB l.c. or alkali 1 inhibited the subsequent binding of [^{125}I]alkali 1 (data not shown).

An estimate of the amount of cross-reactive antibody was made as follows: antiserum to DTNB l.c., pooled from several bleeds of one rabbit, was adsorbed on an affinity column containing 60 mg of chicken DTNB l.c. coupled to Sepharose 4B. Elution with 4 M Gdn·HCl yielded 62 mg of antibody from 50 mL of applied serum. In a parallel experiment, adsorption of 50 mL of anti-DTNB l.c. serum by 60 mg of chicken alkali 1 coupled to Sepharose 4B yielded 18 mg of antibody. The adsorbed sera were recycled through the original immunoadsorbents, with elution yielding less than 5% of that obtained on the first passage. Application of the alkali 1 adsorbed serum to DTNB l.c.–Sepharose allowed recovery of the remaining 40 mg of antibody specific for DTNB l.c. In a control experiment, passage of 50 mL of non-immune serum through a Sepharose-coupled light chain affinity column resulted in nonspecific adsorption of about 3 mg of protein. In summary, antibody cross-reactive with alkali 1 accounted for approximately 25% of the total anti-DTNB l.c. present in the serum.[2]

A third lot of the same serum was adsorbed on rabbit troponin C coupled to Sepharose, with elution yielding up to 5% of the anti-DTNB l.c. present. That the eluted material contained specific antibody was demonstrated by its ability to bind chicken alkali 1, rabbit troponin C, and DTNB l.c. in radioimmunoassays.

Antisera prepared against alkali 1 also contained cross-reactive antibody. Anti-alkali 1 immunoglobulin from two rabbits bound [^{125}I]DTNB l.c. in radioimmunoassays. In both

[2] The low concentration of cross-reactive antibody explains, in part, why alkali 1 is such a poor competitor of the homologous anti-DTNB l.c. reaction (Figure 6a). This result is not inconsistent, however, with the binding data in Figure 3, which imply a high affinity constant for the interaction of alkali 1 with anti-DTNB l.c. antibody. As long as alkali 1 has a lower affinity for anti-DTNB l.c. than does DTNB l.c., it will of necessity compete less effectively for cross-reactive antibody.

cases, the binding of heterologous antigen was substantially weaker than that of immunogen, requiring an approximately 100-fold higher concentration of antibody. The accompanying paper (Holt and Lowey, 1977) describes an unexpected reaction between DTNB l.c. and a population of anti-alkali 1 antibody specific for antigenic determinants within the 41 N-terminal residues unique to alkali 1 (Frank and Weeds, 1974).

Discussion

The sequences of rabbit troponin C (Collins et al., 1973; Collins, 1974), alkali 1 (Frank and Weeds, 1974; Weeds and McLachlan, 1974), DTNB l.c. (Collins, 1976), and scallop EDTA l.c. (Kendrick-Jones and Jakes, 1976) can be aligned with that of the carp calcium-binding protein, parvalbumin (Coffee and Bradshaw, 1973), in such a way as to match approximately 30% of the residues. This limited homology, together with the presence of conservative amino acid substitutions among the intervening nonidentical sequences, allows one to build models of these molecules based on the known structure of parvalbumin (Kretsinger and Nockolds, 1973). Immunological cross-reactivity might be expected among such a group of conformationally similar proteins. Antibody binding studies of cytochromes from phylogenetically diverse sources (Margoliash et al., 1970) and of lysozymes from avian species (Prager and Wilson, 1971) provide a basis for correlation of immunological cross-reactivity with degree of amino acid substitution. Prager notes that proteins differing from each other by 40% or more in sequence exhibit no cross-reactivity. However, this correlation was based on serological reactions that require multivalent antigens, whereas the radioimmunoassay is capable of detecting univalent as well as multivalent antigens. The cross-reactivity we observed is thus probably due to the increased sensitivity of the radioimmunoassay over techniques that depend on precipitation of antibody–antigen complexes.

Other studies of immunological cross-reactivity (for review, see Crumpton, 1974) have shown that antigenicity depends on protein conformation as well as primary structure. Thus, antibodies formed by immunization with denatured protein may not react with native protein. Also, two proteins that contain identical sequences may not share an antigenic determinant unless the sequences occur in a similar secondary structure. For example, Sela et al. (1967) showed that antibodies elicited by immunization with an α-helical polymer of tyrosine-alanine-glutamine did not react with a polylysine polymer that contained tyrosine-alanine-glutamine branches.

Immunological cross-reactivity among myosin light chains and troponin C, therefore, suggests the presence of conformational similarities. However, the failure of heterologous antigens to inhibit reactions between homologous antigens and antibodies demonstrates that the cross-reactivity observed is quite limited. The lack of competition indicates that either a very small percentage of the antibodies is directed against common determinants, or, that the antigenic determinants of the heterologous proteins are sufficiently altered so as to bind antibody with markedly lower affinity. That at least some of these proteins have evolved different shapes is suggested by recent hydrodynamic measurements indicating an asymmetric structure for molluscan light chains (Stafford and Szent-Györgyi, 1976), whereas troponin C and parvalbumin appear to be globular proteins (Kretsinger and Barry, 1975).

We did not observe differences in antigenicity between regulatory and nonregulatory light chains. In fact, the vertebrate myosin light chains showed the most cross-reactivity; approximately one-fourth of the antibodies against DTNB l.c. also recognize alkali 1. The cross-reactivity observed is unlikely to be an artifact due to contaminating heterologous antigen in the immunogen, which could have elicited production of antibody. Purification of antibody by affinity chromatography did not change the extent of cross-reactivity. Moreover, both alkali 1 and DTNB l.c. were able to inhibit the reaction of anti-DTNB l.c. and labeled alkali 1, demonstrating that these antigens are combining with the same antibodies.

As shown in the accompanying paper (Holt and Lowey, 1977), the antigenic determinants shared by alkali 1 and DTNB l.c. appear to include the N-terminal region of these molecules. Antibody to the 41-residue "difference peptide" unique to alkali 1 has to be absorbed with DTNB l.c. before it can separate alkali 1 containing myosin from alkali 2 myosin. Although sequence studies have suggested some degree of structural homology among myosin light chains, the immunological approach used here has emphasized the similarity between the two classes of vertebrate myosin light chains. Perhaps the shared determinants reflect a common role that is unrelated to the ability to restore calcium sensitivity to desensitized scallop myofibrils. Since so little is known at present about the role of the light chains in vertebrate skeletal muscle, a similar function cannot be excluded.

Acknowledgments

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References

Burridge, K., and Bray, D. (1975), *J. Mol. Biol. 99,* 1–14.
Coffee, C. J., and Bradshaw, R. A. (1973), *J. Biol. Chem. 248,* 3305–3312.
Collins, J. H. (1974), *Biochem. Biophys. Res. Commun. 58,* 301–308.
Collins, J. H. (1976), *Nature (London) 259,* 699–700.
Collins, J. H., Potter, J. D., Horn, M. J., Wilshire, G., and Jackman, N. (1973), *FEBS Lett. 36,* 268–272.
Crumpton, M. J. (1974), in The Antigens, Vol. II, Sela, M., Ed., New York, N.Y., Academic Press, pp 1–78.
Ebashi, S., Endo, M., and Ohtsuki, I. (1969), *Q. Rev. Biophys. 2,* 351–384.
Elliott, A., Offer, G., and Burridge, K. (1976), *Proc. R. Soc. London, Ser. B 193,* 45–53.
Frank, G., and Weeds, A. G. (1974), *Eur. J. Biochem. 44,* 317–334.
Holt, J. C., and Lowey, S. (1975), *Biochemistry 14,* 4600–4609.
Holt, J. C., and Lowey, S. (1977), *Biochemistry 16* (preceding paper in this issue).
Holtzer, A., and Lowey, S. (1959), *J. Am. Chem. Soc. 81,* 1370–1377.
Hunter, W. M. (1970), in Handbook of Experimental Immunology, 2nd ed, Weir, D. M., Ed., Oxford, England, Blackwell Scientific Publications, p 17.19.
Kendrick-Jones, J. (1974), *Nature (London) 249,* 631–634.
Kendrick-Jones, J., and Jakes, R. (1977), in International Symposium on Myocardial Failure at Tegernsee, Rieker, G., and Boehringer, Ed., Munich, West Germany, Springer-Verlag, pp 28–40.
Kendrick-Jones, J., Lehman, W., and Szent-Györgyi, A. G. (1970), *J. Mol. Biol. 54,* 313–326.

Kendrick-Jones, J., Szentkiralyi, E. M., and Szent-Györgyi, A. G. (1976), *J. Mol. Biol. 104,* 747-775.
Kretsinger, R. H., and Barry, C. D. (1975), *Biochim. Biophys. Acta 405,* 40-52.
Kretsinger, R. H., and Nockolds, C. E. (1973), *J. Biol. Chem. 248,* 3313-3326.
Kuettner, M. G., Wang, A. L., and Nisonoff, A. (1972), *J. Exp. Med. 135,* 579-595.
Lowey, S., and Risby, D. (1971), *Nature (London) 234,* 81-85.
Margoliash, E., Nisonoff, A., and Reichlin, M. (1970), *J. Biol. Chem. 245,* 931-939.
Omenn, G. S., Ontjes, D. A., and Anfinsen, C. B. (1970), *Nature (London) 225,* 189-190.
Prager, E. M., and Wilson, A. C. (1971), *J. Biol. Chem. 246,* 5978-5989.
Sela, M., Schechter, B., Schechter, I., and Borek, F. (1967), *Cold Spring Harbor Symp. Quant. Biol. 32,* 537-545.
Stafford, W. F., III, and Szent-Györgyi, A. G. (1976), *Biophys. J. 16,* 70a.
Squire, J. M. (1975), *Annu. Rev. Biophys. Bioeng. 4,* 137-163.
Szent-Györgyi, A. G., Szentkiralyi, E. M., and Kendrick-Jones, J. (1973), *J. Mol. Biol. 74,* 179-203.
Weber, K., and Osborn, M. (1969), *J. Biol. Chem. 244,* 4406-4412.
Weeds, A. G., and McLachlan, A. D. (1974), *Nature (London) 252,* 646-649.

Optical Activity of Disulfide Bonds in Proteins: Studies on Human Choriomammotropin and Bovine Pituitary Somatotropin†

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ABSTRACT: The contributions of the homologous carboxyl-terminal disulfide bonds in human choriomammotropin (human chorionic somatomammotropin, human placental lactogen) and bovine somatotropin (pituitary growth hormone), to the near ultraviolet circular dichroism spectra of these two proteins have been evaluated. The disulfide bond in the human placental protein displays a broad, negative band centered near 260 nm ($[\theta]_{M,\,260nm} = -2100 \pm 160$ deg cm^2 dmol^{-1}) which is equivalent, within experimental error, to the band previously assigned to the identical disulfide in plasmin modified human somatotropin. The homologous disulfide in the bovine hormone also exhibits a negative band, very similar in intensity ($[\theta]_{M,\,254nm} = -2200 \pm 210$ deg cm^2 dmol^{-1}), with an estimated band center blue-shifted relative to the human proteins to 252-255 nm. Reoxidation of either partially reduced protein results in complete repair of the circular dichroism spectrum to that of the native protein. No definite contributions could be assigned below 240 nm to the optical activity of these disulfide bonds. Circular dichroism measurements have also been used to approximate the rates of reduction of the two proteins.

In a previous publication (Bewley, 1977) the individual contributions of each of the two disulfide bonds in plasmin modified somatotropin (PL-HGH)[1] to the total optical activity of the protein were estimated from circular dichroism spectra of a series of partially and completely reduced, as well as partially and completely reduced-carbamidomethylated derivatives of the hormone. It was found that the disulfide bond forming the smaller eight-membered loop near the carboxyl terminus of the molecule could be selectively and quantitatively reduced with DTT in the absence of denaturants without loss of biological or immunological activities (Li and Bewley, 1976). Within experimental error, no differences were found in the CD spectra of the unmodified and modified forms of the protein in the region of amide bond absorption. In the region of side-chain absorption, the reduced and reduced-carbamidomethylated derivatives appeared to fully retain the CD bands previously assigned to tryptophan, tyrosine, and phenylalanine chromaphores in PL-HGH and HGH. However, the CD spectra of the derivatives lacking the carboxyl-terminal disulfide bond were slightly shifted toward less negative ellipticity values between 285 and 245 nm, relative to PL-HGH. The maximum shift appeared around 258 nm. Subtraction of the CD spectrum of the partially reduced or partially reduced-carbamidomethylated derivative from that of PL-HGH resulted in a weak, broad, negative CD band centered near 258 nm ($[\theta]_{M,\,258nm} = -2100$ deg cm^2 dmol^{-1}) which was assigned to the optical activity of the disulfide bond linking half-cystine residues 182 and 189. Reoxidation of the partially reduced protein produced a complete repair of the side-chain CD spectrum. In a similar manner the second disulfide bond, linking half-cystine residues 53 and 165, could also be reduced and carbamidomethylated without loss of biological activity or change in the far-UV CD spectrum (Li and Bewley, 1976; Bewley, 1977). In the region of side-chain absorption the CD of the completely reduced-carbamidomethylated derivative again appeared to largely retain the bands originating from the aromatic chromophores in PL-HGH. Subtraction of this spectrum from that of the partially reduced-carbamidomethylated derivative produced a second broad, negative CD band, somewhat more intense than the first with its center red-shifted to around 273 nm ($[\theta]_{M,273nm} = -3200$ deg cm^2

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[1] Abbreviations used are: PL-HGH, human pituitary growth hormone modified by limited digestion with human plasmin; HGH, human pituitary growth hormone; HCS, human choriomammotropin (human chorionic somatomammotropin, human placental lactogen); BGH, bovine pituitary growth hormone; o-PR, ovine pituitary prolactin; DTT, dithiothreitol; CD, circular dichroism; UV, ultraviolet; SE, standard error.