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The interaction of actin with dystrophin

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Proton NMR spectroscopy of synthetic peptides corresponding to defined regions of human dystrophin has been employed to study the interaction with F-actin. No evidence of interaction with a C-terminal region corresponding to amino acid residues 3429–3440 was obtained. F-actin restricted the mobility of residues 19–27 in a synthetic peptide corresponding to residues 10–32. This suggests that this is a site of F-actin interaction in the intact dystrophin molecule. Identical sequences to that of residues 19–22 in dystrophin, namely Lys-Thr-Phe-Thr are also present in the N-terminal regions of the α-actinins implying this is also a site of F-actin interaction with α-actinin.

Proton nuclear magnetic resonance; Dystrophin; F-actin; Interaction; α-Actinin; Synthetic peptide

1. INTRODUCTION

The recent work of the Kunkel [1] and Worton [2] groups has established that dystrophin, a protein of predicted molecular weight 427000, is absent or in some cases present in modified form in patients with Duchenne and Becker muscular dystrophies (Xp21 myopathies). Study of the amino acid sequence of dystrophin suggests that four domains can be distinguished in the molecule [3]. These are the N-terminal region possessing homologies with α-actinin, a long run of triple helical segments resembling spectrin, a cysteine-rich sequence and a C-terminal domain. Although it has been presumed from the homologies with α-actinin that actin interacts with the N-terminal domain of dystrophin, direct evidence of this has not yet been reported. Proton NMR spectroscopy applied to defined protein and peptide sequences is a powerful tool for the investigation of protein interactions, as we have demonstrated in earlier studies on the interaction of actin with troponin I [4] and caldesmon [5]. By extending this approach to dystrophin, we have identified one site on this protein that is involved in interaction with actin. Our observations provide insight into the functional aspects of the N-terminal region of dystrophin, a region shown to be retained in the shortened form of the molecule found in patients with mild forms of Xp21 myopathy [6].

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2. MATERIALS AND METHODS

Peptides were synthesized by Alta Bioscience, Birmingham University using Fmoc polyamide chemistry, purified by HPLC and finally treated with glacial acetic acid for 2 h at 18°C to remove traces of blocking agents. Acetic acid was removed by freeze-drying followed by repeated dissolution in water and freeze-drying. Actin from rabbit skeletal muscle was prepared as described previously [4]. Samples were dissolved in D_2O and proton NMR spectroscopy was carried out on a Brüker 500 MHZ instrument operated under the auspices of the Oxford Centre for Molecular Sciences. Titrations with actin were carried out by the addition of small aliquots of a stock solution (200 μM) containing 0.5 mM DTT. Two dimensional COSY experiments were carried out as previously described [7].

3. RESULTS AND DISCUSSION

In our initial studies, the possible interaction of actin with two synthetic peptides, namely those corresponding to residues 1–32 and 3429–3440 of human dystrophin, was investigated. The C-terminal peptide was readily soluble, but on addition of F-actin up to a 2:1 molar excess to a solution containing 0.4 mM peptide in 0.5 mM DTT, 10 mM sodium bicarbonate, pH 7.0, the spectra obtained corresponded to the sums of the components. Thus, there was no evidence of interaction.

The N-terminal peptide was much less soluble than that from the C-terminus but 100 μM solutions that gave satisfactory signals were obtained in 0.5 mM DTT, 10 mM sodium bicarbonate, pH 7.0. Peptide 1–32 appeared to be aggregated in solution as judged by the extensively broad appearance of the signals, particularly those due to hydrophobic residues (Fig. 1).

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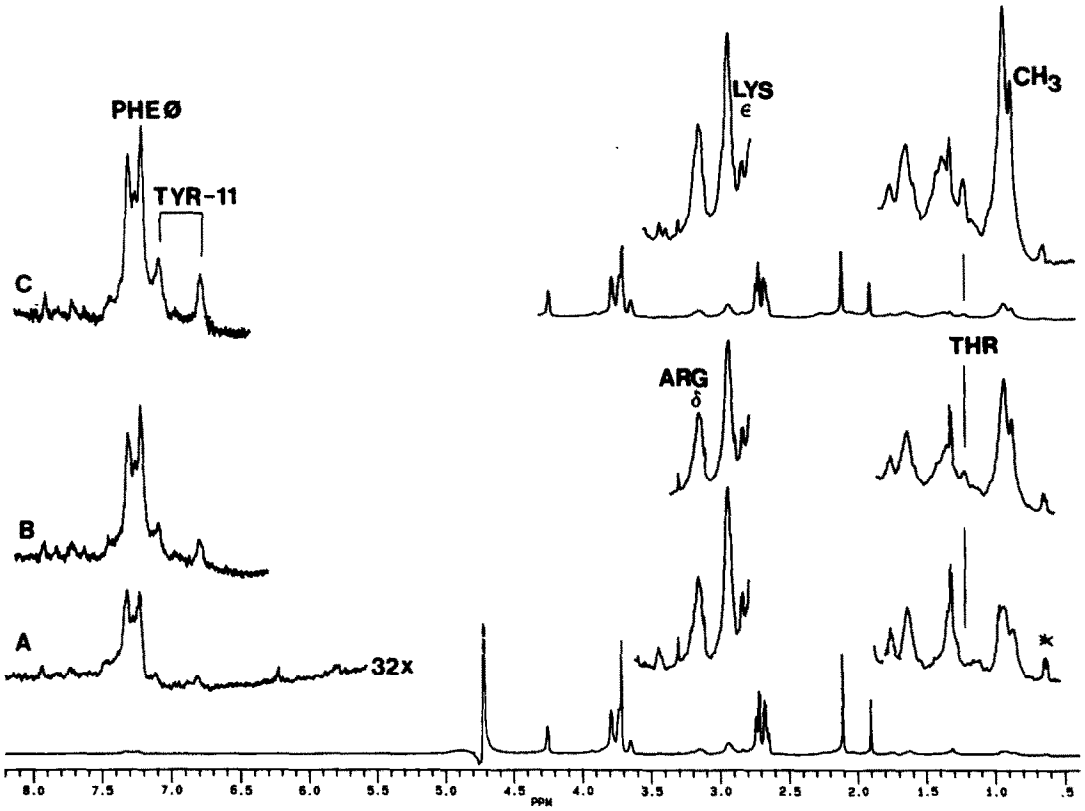

Fig. 1. ^{1}H-NMR spectral effects of the binding of F-actin to dystrophin residues 1–32. (A) Dystrophin peptide, 160 μM, in D_2O solution. Note the very broad lineshapes of the peptide resonances shown on an expanded scale (32 ×). (B) Addition of 8 μM F-actin causes an apparent increase in signal intensity (cf. the signal of the standard denoted by a *) resulting from a decrease in resonance line width. (C) In the presence of 16 μM F-actin, the resonances of, e.g. Tyr-11 and Thr-20,22 are now readily resolved.

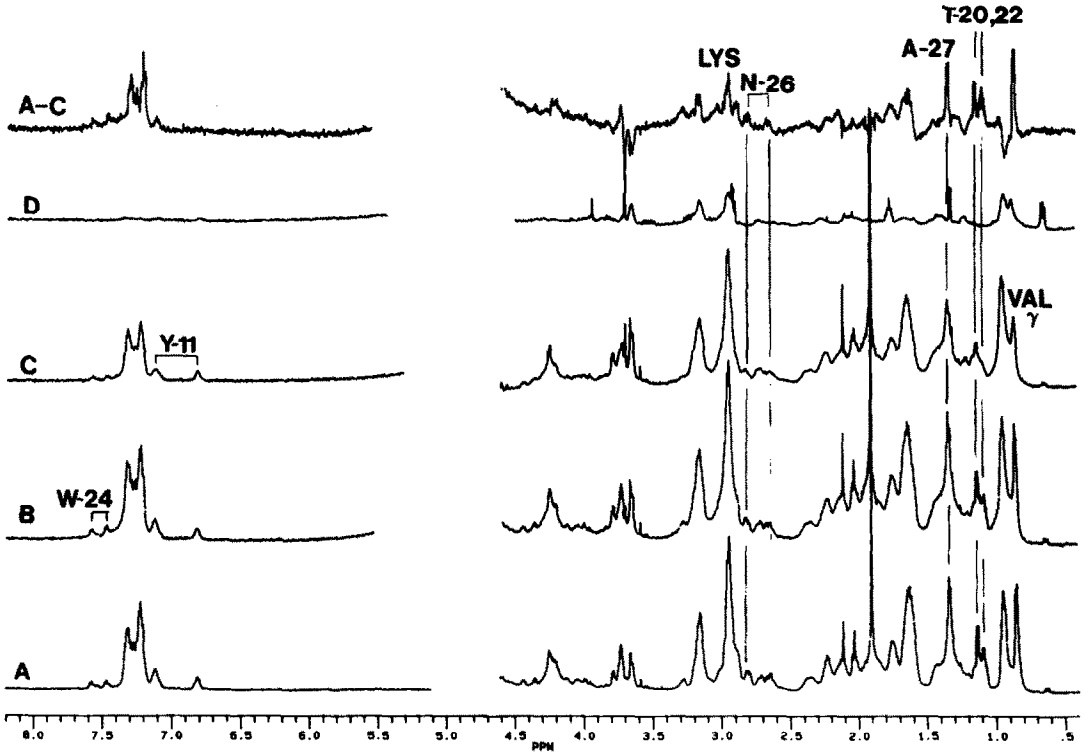

Fig. 2. ^{1}H-NMR spectral titration of dystrophin residues 10–32 with F-actin. (A) 200 μM dystrophin peptide. (B) and (C) upon addition of 8 μM and 16 μM F-actin, respectively (cf. Fig. 1). (D) Spectrum of the stock solution of F-actin titrated into the peptide. (A–C) Difference spectrum resolving signals of the peptide perturbed by complex formation with F-actin. The resonance assignments shown were obtained by analysis of the COSY spectrum (Fig. 3).

The signal intensities did not correspond well to the composition since the majority did not integrate properly indicating multimeric aggregates.

On the addition to peptide 1–32 of F-actin up to 16 μM, at which concentration actin does not contribute significantly to the spectrum, marked changes in the peptide spectrum occurred progressively as the actin concentration increased (Fig. 1). The signal intensities due to the CH_3 of valine and leucine, the γ-CH_3 of threonine and those due to aromatic residues increased. This implies that disaggregation of the peptide has occurred, given the observed increase in mobility now available to these groups. Addition of actin also led to some precipitate formation. The observations suggested that actin interacts with peptide 1–32 with an affinity constant $>10^4$, given the concentrations used, but the interpretation of the NMR spectra is complicated by the disaggregation of the peptide as a consequence of the interaction.

The signals from tryptophan and tyrosine residues that are concentrated in the N-terminal part of peptide 1–32 were broadened in the absence of actin, whereas those arising from phenylalanine in the C-terminal part were not. This suggests that the aggregation involved in

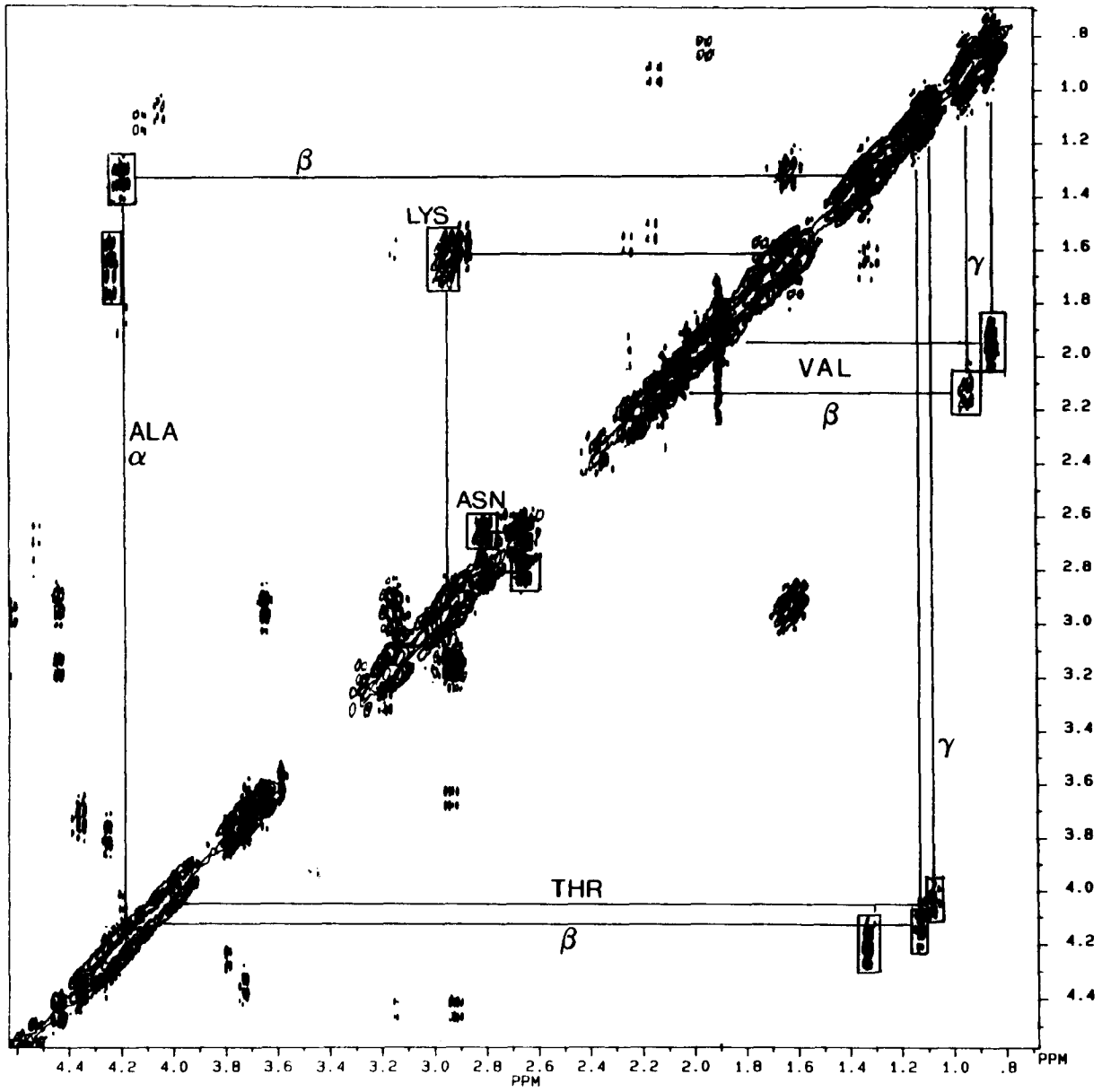

Fig. 3. ^{1}H homonuclear correlated contour plot of the dystrophin peptide, residues 10–32, showing the chemical shift positions of through-bond correlated resonances. The resonances identified in Fig. 2 are boxed. Peptide concentration 300 μM, pH 7.2, T = 300 K. The spectrum was obtained with 400 increments each of size 2K with 64 transients for each value of t_1 and a relaxation delay of 1 s. After acquisition, the data were multiplied by a sine-bell function and the 2-dimensional transform processed to a final size of 2K by 2K.

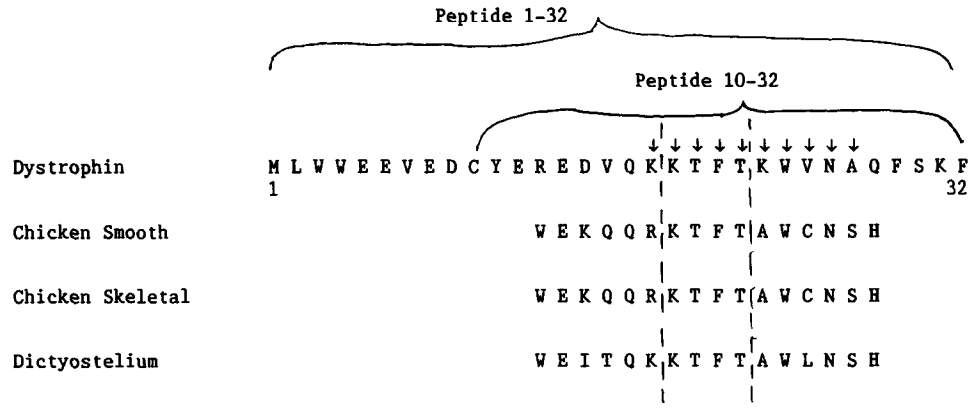

Fig. 4. N-terminal sequences of human dystrophin and the α-actinins. Brackets indicate N-terminal peptides used in NMR studies. Sequences from Blanchard et al. [8]. Residues of the dystrophin N-terminal sequence that are perturbed on interaction with F-actin are indicated by arrows.

particular the N-terminal region of the peptide. An additional peptide corresponding to residues 10–32 of dystrophin was synthesized and examined in the expectation that aggregation would be minimized and facilitate the interpretation of the effects with actin. Fig. 2 shows that well-resolved signals occur for all the residues, indicating that peptide 10–32 is not aggregated. The coupling patterns readily discernible for the various proton resonances enabled us to go on to examine the peptide spectrum by two-dimensional correlated spectroscopy (Fig. 3) to derive the assignments shown in Fig. 2.

Addition of F-actin to peptide 10–32 indicated that selective broadening of the specific signals, e.g. due to phenylalanine, occurred progressively during titration. The differential perturbation of the various peptide group signals is apparent from the difference spectra shown in Fig. 2. This indicates that the resonances of the β-CH_3 of alanine 27, the γ-CH_3 of threonines 20 and 22, the ϵ-CH_2 of lysine and the γ-CH_3 of one of the two valines but not of tyrosine were perturbed by the addition of F-actin. Similar results were obtained with three different actin preparations. In the absence of aggregation of peptide 10–32, it can be concluded that changes in signals observed reflect specific interactions of actin with the peptide. In view of the absence of evidence for interaction with the C-terminal dystrophin peptide and our experience of the validity of extrapolating the results obtained with peptides to the whole molecule from which they were derived [4,5], we conclude that the region represented by residues 10–32 in the intact dystrophin molecule encompasses a site of interaction with actin.

When putative actin-binding domains of four different α-actinins and human dystrophin are aligned (Fig. 4) the residues at positions 19–22 in the latter protein, namely Lys-Thr-Phe-Thr, are identical in all five proteins [7]. We believe that it is highly significant that the NMR studies indicate that these residues are perturbed when actin interacts with peptide 10–32. Although these investigations indicate that residues 19–22 together with associated residues are involved in interaction with actin, we are unable to say yet whether this is the only site of interaction between these two proteins. It might be presumed that these residues are also involved in the interactions of the α-actinins with F-actin. In view of the basic nature of this actin contact segment of dystrophin and hence its similarity to the actin contact regions of both troponin I and caldesmon [4,5], it is plausible that binding occurs at or close to the acidic N-terminal residues of actin.

Work is in progress to determine whether other regions of the dystrophin molecule are involved in interaction with actin and to define regions of the actin amino acid sequence that are concerned.

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