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Z-band Alternatively Spliced PDZ Motif Protein (ZASP) Is the Major *O*-Linked β-*N*-Acetylglucosamine-substituted Protein in Human Heart Myofibrils*[S]

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Background: We studied *O*-GlcNAc-modified protein in sarcomeric proteins of human heart.
Results: ZASP (Z-band alternatively spliced PDZ motif protein) accounts for 50–80% of *O*-GlcNAcylated protein.
Conclusion: ZASP is the major *O*-GlcNAc-substituted protein in human heart muscle, and its levels increase in pathological muscle.
Significance: Modulation of *O*-GlcNAcylation in ZASP may have a role in mechanotransduction in the heart.

We studied *O*-linked β-*N*-acetylglucosamine (*O*-GlcNAc) modification of contractile proteins in human heart using SDS-PAGE and three detection methods: specific enzymatic conjugation of *O*-GlcNAc with UDP-*N*-azidoacetylgalactosamine (UDP-GalNAz) that is then linked to a tetramethylrhodamine fluorescent tag and CTD110.6 and RL2 monoclonal antibodies to *O*-GlcNAc. All three methods showed that *O*-GlcNAc modification was predominantly in a group of bands ~90 kDa that did not correspond to any of the major myofibrillar proteins. MALDI-MS/MS identified the 90-kDa band as the protein ZASP (Z-band alternatively spliced PDZ motif protein), a minor component of the Z-disc (about 1 per 400 α-actinin) important for myofibrillar development and mechanotransduction. This was confirmed by the co-localization of *O*-GlcNAc and ZASP in Western blotting and by immunofluorescence microscopy. *O*-GlcNAcylation of ZASP increased in diseased heart, being 49 ± 5% of all *O*-GlcNAc in donor, 68 ± 9% in end-stage failing heart, and 76 ± 6% in myectomy muscle samples (donor *versus* myectomy $p < 0.05$). ZASP is only 22% of all *O*-GlcNAcylated proteins in mouse heart myofibrils.

The post-translational modification, *O*-linked β-*N*-acetylglucosamine (*O*-GlcNAc),[2] on nuclear and cytoplasmic proteins has attracted a lot of interest since it was first described in 1984 (1). Analogous to phosphorylation, *O*-GlcNAcylation occurs on serine and threonine residues, and cross-talk between protein *O*-GlcNAcylation and phosphorylation has been proposed to play a role in signaling and transcription regulation (2).

A role for protein *O*-GlcNAcylation has been proposed in regulating growth and contractility in the mammalian heart. Global *O*-GlcNAc levels in the heart have been observed to increase in rats with hypertension, surgically induced hypertrophy, and heart failure and also in heart tissue from patients with aortic stenosis (3). It is interesting to note that there was a decrease in *O*-GlcNAc in physiologically hypertrophied heart in a study using swim-exercised mice (4) and that short term *O*-GlcNAcylation affects myofibrillar Ca^{2+} sensitivity (5) and can be cardioprotective (6). It was suggested these effects involved *O*-GlcNAcylation of sarcomeric proteins.

Most investigations have measured total *O*-GlcNAc in heart muscle, and identification of the proteins of the contractile apparatus that are *O*-GlcNAc-substituted has been limited to animal studies. *O*-GlcNAc modifications on most of the major myofibrillar proteins have been reported in rat cardiac and skeletal muscles, including myosin heavy chain, actin, troponin I, myosin light chain 1, and myosin light chain 2, but stoichiometry of the modification was not quantified (7–9).

Here, we have studied *O*-GlcNAc modifications of myofibrillar proteins in human heart muscle myofibrils for the first time. Using three independent measurement techniques, we found that, unlike rat and mouse, the majority of *O*-GlcNAc in human myofibrils was in a low abundance protein, identified as ZASP (Z-band alternatively spliced PDZ motif protein, also named LIM domain-binding protein 3, Cypher, and Oracle (*LDB3* gene)) (10). We also observed an apparent increase in *O*-GlcNAc modification of ZASP in pathological human heart samples.

EXPERIMENTAL PROCEDURES

Human Cardiac Samples—Cardiac muscle from donor hearts as control, end-stage failing hearts, and septal myectomy samples from patients with hypertrophic obstructive cardiomyopathy were obtained as described (11–14) (and

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[S] This article contains supplemental text and Tables 1–3.

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[2] The abbreviations used are: *O*-GlcNAc, *O*-linked β-*N*-acetylglucosamine; GalNAz, *N*-azidoacetylgalactosamine; ZASP, Z-band alternatively spliced PDZ motif protein; ENH, enigma homologue; TAMRA, tetramethylrhodamine; cMyBP-C, cardiac myosin-binding protein-C.

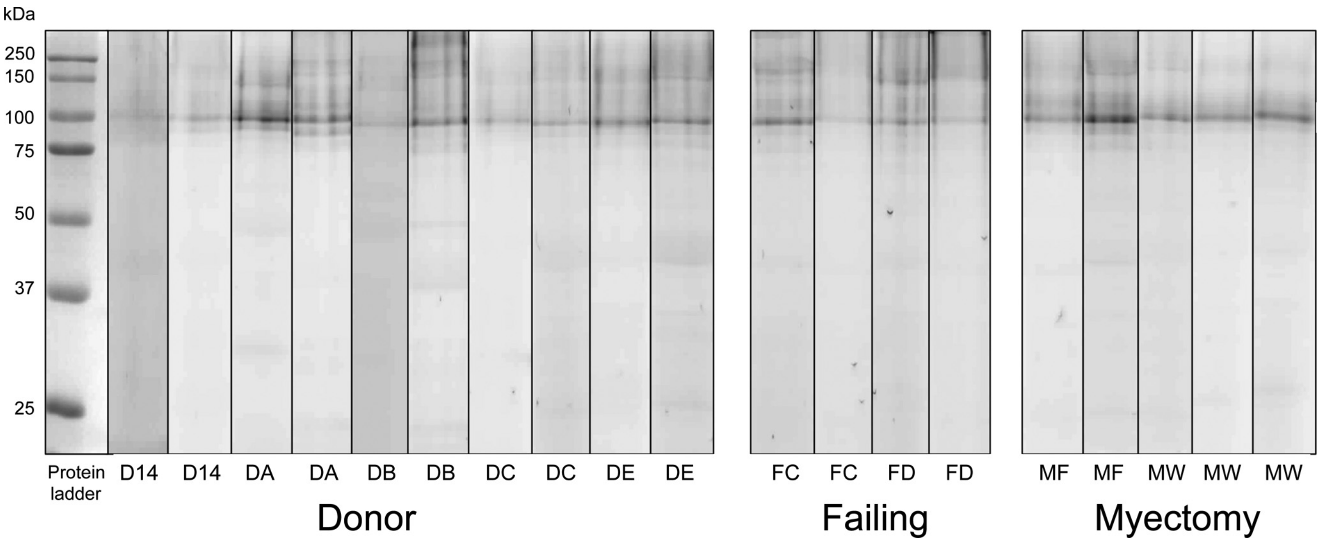


FIGURE 1. **SDS-PAGE gels of myofibrillar proteins (4 μg) following specific enzymatic labeling of *O*-GlcNAc visualized by TAMRA fluorescence.** Inverted images are shown here. Duplicate assays of myofibrils from five donor hearts (*D*), two failing hearts (*F*), and two myectomy samples (*M*) were performed. *Numbers* and *letters* identify the samples that have been described in previous studies (12–14, 38–40).

see supplemental text). All tissues were rapidly frozen and stored under liquid nitrogen until use. Gender, age, and known mutations of subjects are presented in supplemental Table 1.

Enzymatic Labeling of O-GlcNAcylated Proteins—Human heart myofibrils and cardiac tissue whole lysates were isolated as described (13–15). Proteins from myofibril extracts were precipitated by chloroform/methanol precipitation and resuspended into 20 mM HEPES buffer with 1% SDS, pH 7.9. *O*-GlcNAc groups were labeled using the Click-iT^{TM} *O*-GlcNAc enzymatic labeling system from Invitrogen. Procedures from the manufacturer's protocol were followed (16, 17). Briefly, the sample was mixed with distilled H_2O, Click-iT^{TM} *O*-GlcNAc enzymatic labeling buffer, and $MnCl_2$ solution and vortexed. UDP-GalNAz was then added followed by the addition of the genetically modified enzyme Gal-T1 (Y289L), which puts the GalNAz onto any *O*-GlcNAc groups. This was incubated overnight at 4 °C. Chloroform/methanol precipitation was performed, and the proteins were resuspended in 50 μl of 50 mM Tris-HCl, 1% SDS, pH 8.0. Detection was enabled using Click-iT^{TM} tetramethylrhodamine (TAMRA) protein analysis detection kit from Invitrogen following the manufacturer's instructions. Briefly, reaction buffer, containing TAMRA conjugated with an alkyne group, was mixed with the resuspended sample followed by the addition of distilled H_2O, $CuSO_4$ solution, and two other reaction buffers. This mixture was vortexed for 20 min for the Click-iT^{TM} azide/alkyne reaction to attach the TAMRA label to the GalNAz-labeled *O*-GlcNAc groups. Further chloroform/methanol precipitation was then performed, and the protein was resuspended in SDS buffer. Labeled samples were used immediately.

SDS-PAGE and Western Blotting—Myofibril proteins labeled with TAMRA enzymatic labeling were separated using 12% SDS-PAGE. The TAMRA fluorescence signal was visualized with UV transillumination, and the image was recorded with a CCD camera-based gel imager (G:BOX, Syngene) and analyzed using GeneTools (Syngene).

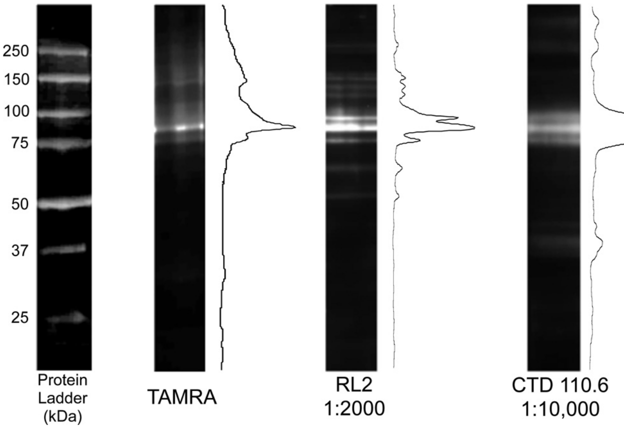
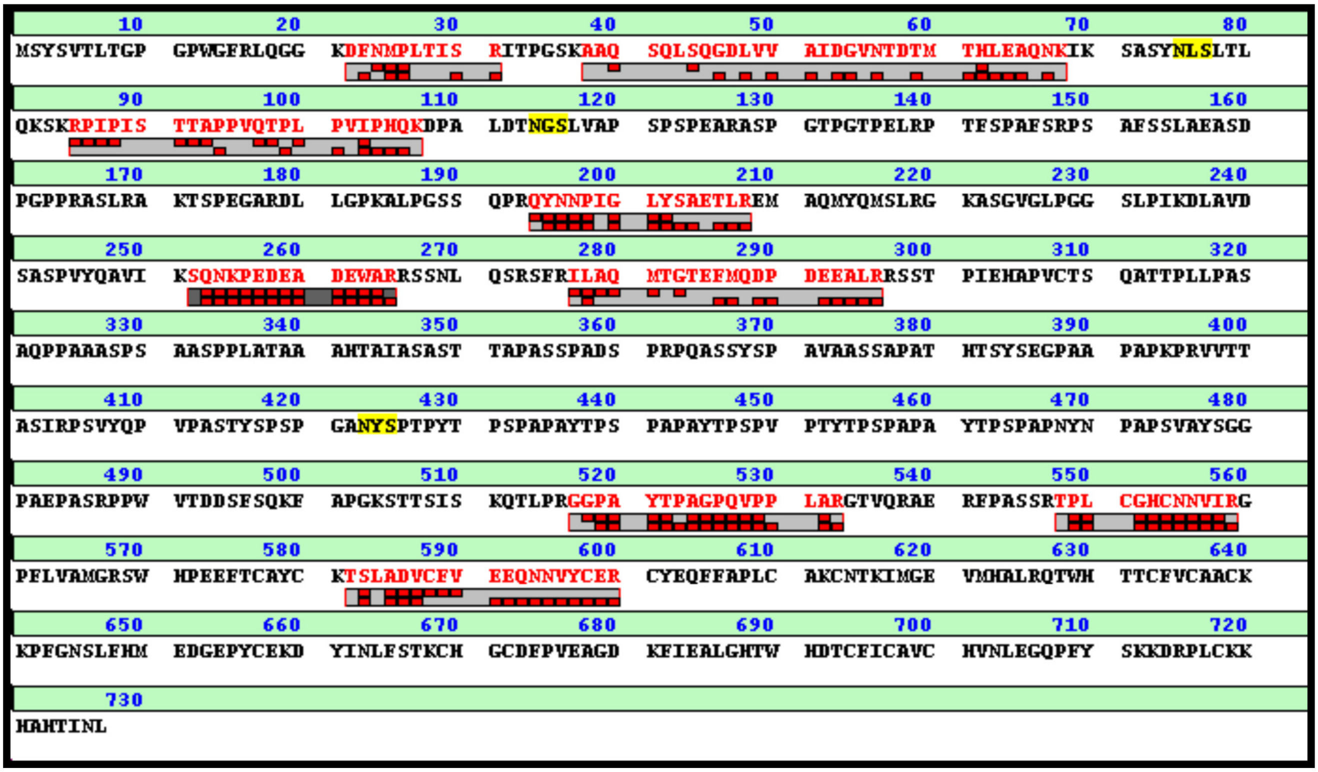


FIGURE 2. **Comparison of detection of *O*-GlcNAc in donor heart myofibrils (4 μg) separated by SDS-PAGE and visualized by enzymatic labeling (TAMRA fluorescence) and Western blots using RL2 and CTD110.6 primary antibodies (detected by chemiluminescence).** Original gel and corresponding densitometer traces are shown. Bands in the 90-kDa region predominated with all three detection methods.

Myofibril samples were separated on 10% SDS-PAGE gels and Western blotted. Proteins were transblotted onto nitrocellulose membrane. Nitrocellulose membranes with the transferred proteins were cut into strips so that each strip had the same protein profile. The strips were probed separately with different antibodies and then reassembled in register for chemiluminescent detection. Primary antibodies used were as follows: for *O*-GlcNAc, CTD110.6 antibody to *O*-GlcNAc, a kind gift from Gerald Hart, Johns Hopkins University School of Medicine, Baltimore, MD; and monoclonal antibody (RL2), from Thermo Scientific; for ZASP, LDB3 antibody NB100-2445, from Novus Biologicals; and for enigma homologue (ENH), PDLIM5 (JK-3R), from Santa Cruz Biotechnology.

Antibody dilutions are indicated in the corresponding figures. Secondary antibodies were the appropriate horseradish peroxidase-conjugated IgGs (see supplemental text), and detection was by chemiluminescence using ECL Plus Western



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        10         20         30         40         50         60         70         80
MSYSVTLTGP GPWGFRLQGG KDFNMPLTIS RITPGSKAAQ SQLSQGDLVV AIDGVNTDTM THLEAQNKIK SASYNLSLTL
        90        100        110        120        130        140        150        160
QKSKRPIPIS TTAPPVQTPL PVIPHQKDPA LDTNGSLVAP SPSPEARASP GTPGTPELRP TFSPAFSRPS AFSSLAEASD
       170        180        190        200        210        220        230        240
PGPPRASLRA KTSPEGARDL LGPKALPGSS QPRQYNNPIG LYSAETLREM AQMYQMSLRG KASGVGLPGG SLPIKDLAVD
       250        260        270        280        290        300        310        320
SASPVYQAVI KSQNKPEDEA DEWARRSSNL QSRSFRILAQ MTGTEFMQDP DEEALRRSST PIEHAPVCTS QATTPLLPAS
       330        340        350        360        370        380        390        400
AQPPAAASPS AASPPLATAA AHTAIASAST TAPASSPADS PRPQASSYSP AVAASSAPAT HTSYSEGPAA PAPKPRVVTT
       410        420        430        440        450        460        470        480
ASIRPSVYQP VPASTYSPSP GANYSPTPYT PSPAPAYTPS PAPAYTPSPV PTYTPSPAPA YTPSPAPNYN PAPSVAYSGG
       490        500        510        520        530        540        550        560
PAEPASRPPW VTDDSFSQKF APGKSTTSIS KQTLPRGGPA YTPAGPQVPP LARGTVQRAE RFPASSRTPL CGHCNNVIRG
       570        580        590        600        610        620        630        640
PFLVAMGRSW HPEEFTCAYC KTSLADVCFV EEQNNVYCER CYEQFFAPLC AKCNTKIMGE VMHALRQTWH TTCFVCAACK
       650        660        670        680        690        700        710        720
KPFGNSLFHM EDGEPYCEKD YINLFSTKCH GCDFPVEAGD KFIEALGHTW HDTCFICAVC HVNLEGQPFY SKKDRPLCKK
       730
HAHTINL
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FIGURE 3. **Location of peptides in the ZASP 1 amino acid sequence (77.1 kDa).** The 90-kDa band from TAMRA-labeled myofibrils separated on SDS-PAGE, as in Fig. 1, was identified by MALDI-TOF/TOF of trypsin digests (Bruker ultrafleXtreme instrument). Peptide identification was performed using the Mascot search engine, specifying human taxonomy for search in the Swiss-Prot database (versions 83.1). Results of the second analysis are shown. Coverage was 22.1%, and nine unique peptides were identified. The first analysis (AB Scitex 4800 instrument) identified the two unique peptides ^{252}KSQNKPEDEADEWARR^{265} and ^{194}RQYNNPIGLYSAETLRE^{208}.

blotting detection reagents from GE Healthcare. Chemiluminescence was imaged by cooled CCD camera using the G:BOX (Syngene) and densitometry was carried out using GeneTools (Syngene).

Protein Identification by MALDI-TOF/TOF—The TAMRA-labeled 90-kDa protein band was cut out of the gel, and in-gel reduction, alkylation, and tryptic digestion performed. The resulting peptides were fractionated by HPLC, mixed with matrix, and spotted onto target plates for MALDI-TOF/TOF analysis. Protein identification was performed using MASCOT to search the Swiss-Prot human database. Full details are given in the supplemental text.

Double Indirect Immunofluorescence—Snap-frozen cardiac tissues of ~0.5 cm^3 size were embedded in Tissue-Tek OCT compound (Sakura Finetek) and stored at −80 °C. 5-μm-thick sections were cut at −20 °C and stored at −80 °C until use. Sections were fixed in ice-cold methanol for 10 min and rinsed in PBS. They were then incubated with blocking serum (10% goat serum in PBS) for 1 h at room temperature followed by primary antibodies (ZASP, Cypher (H-83), Santa Cruz Biotechnology, dilution 1:50; RL2, dilution 1:200; cMyBP-C (cardiac myosin-binding protein-C), 2-14 antibody (18), dilution 1:500; α-actinin, clone EA-53, Sigma-Aldrich, dilution 1:4000) overnight at 4 °C. The sections were then washed three times for 5 min each in PBS with 0.05% Tween 20 and once for 5 min in PBS alone. Incubation with secondary antibodies (Alexa Fluor® 488 goat anti-rabbit IgG (H+L), highly cross-adsorbed; Alexa Fluor® 555 goat anti-mouse IgG (H+L), highly cross-adsorbed, both from Invitrogen) was for 1 h at room temperature. The sections were then washed as before and coverslipped with mounting medium with 4′,6-diamidino-2-phenylindole for nuclei staining (VECTASHIELD mounting medium with DAPI, Vector Laboratories). Negative controls were conducted by replacing primary antibody and secondary antibody with PBS. Only donor samples were examined due to limited availability of diseased samples.

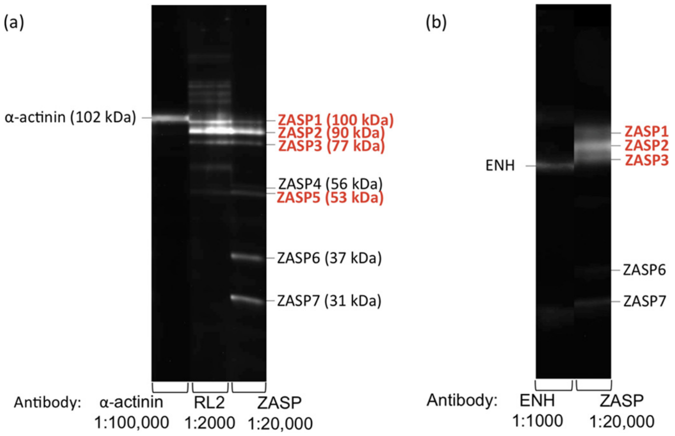

FIGURE 4. **Western blots of donor heart myofibrils (4 μg) separated by SDS-PAGE.** *a*, the gel samples were probed with antibodies specific to *O*-GlcNAc (RL2), ZASP, and α-actinin; ZASP was identified as the major *O*-GlcNAcylated protein, and α-actinin was excluded. *b*, the same gel samples were probed with antibodies specific to ENH and ZASP, showing specificity of the ZASP antibody and excluding ENH as a major *O*-GlcNAcylated protein.

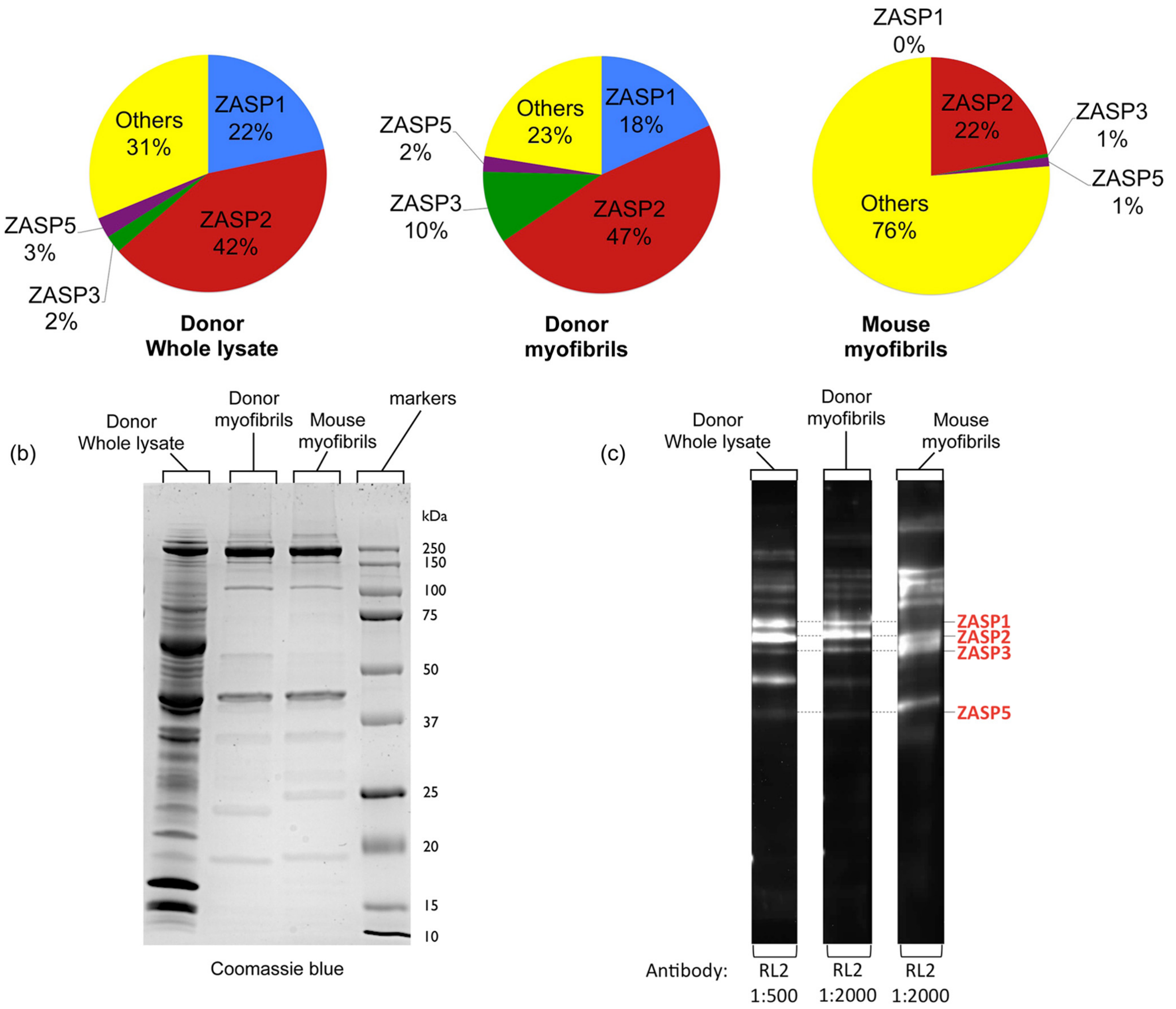

FIGURE 5. **Patterns of *O*-GlcNAcylation in whole human heart lysate, myofibrillar fraction, and mouse myofibrils.** *a*, pie charts showing the distribution of *O*-GlcNAcylated proteins in donor whole lysate and the myofibrillar fraction of donor heart and mouse myofibrils. Individual *O*-GlcNAcylated proteins were detected in Western blots with RL2 antibody (Fig. 2) and expressed as a fraction of total *O*-GlcNAcylated protein. *b*, total protein in donor whole lysate, donor myofibrils, and mouse myofibrils separated by SDS-PAGE and Coomassie Blue staining. *c*, *O*-GlcNAcylated protein in donor whole lysate, donor myofibrils, and mouse myofibrils separated by SDS-PAGE. Western blots were probed with anti-*O*-GlcNAc antibody RL2.

RESULTS

O-GlcNAcylation in Human Cardiac Myofibril Proteins—Results using the highly specific *O*-GlcNAc enzymatic labeling of myofibrils from donor, failing, and myectomy human heart muscle are presented in Fig. 1. In contrast to previous studies on rodent heart myofibrils (7), all the samples showed a predominant *O*-GlcNAc-modified protein at 90 kDa. Two *O*-GlcNAc specific antibodies, CTD110.6 and RL2, were also used to detect *O*-GlcNAcylation in human myofibrils. Both antibodies labeled a predominant group of bands in the 90-kDa region, and the 90-kDa band was the only labeled band common to all three detection methods, indicating that it is highly likely to be *O*-GlcNAc-modified rather than nonspecifically labeled (Fig. 2).

Identification of the 90-kDa Band as ZASP—*O*-GlcNAcylated protein in human heart myofibrillar fractions was enzymatically labeled with TAMRA and separated by SDS-PAGE. The TAMRA-labeled 90-kDa protein band was excised for identification by MALDI-TOF/TOF mass spectrometry. Two separate analyses were performed using different donor heart samples. The possible matches with proteins in the Swiss-Prot human protein database are shown in supplemental Table 2. The list includes most of the components of the sarcomere, possibly due to smearing in the gel because of their abundance. Both analyses indicated the presence of the LIM domain-binding protein 3 (*LDB3* gene, commonly known as ZASP) in the gel band with two and nine unique peptides, respectively (Fig. 3). Only ZASP, the similar Z-disc protein, ENH, and α-actinin have molecular masses close to 90 kDa in the lists of proteins identified.

We confirmed that the 90-kDa protein band is ZASP by Western blotting with specific antibodies (Fig. 4). The ZASP

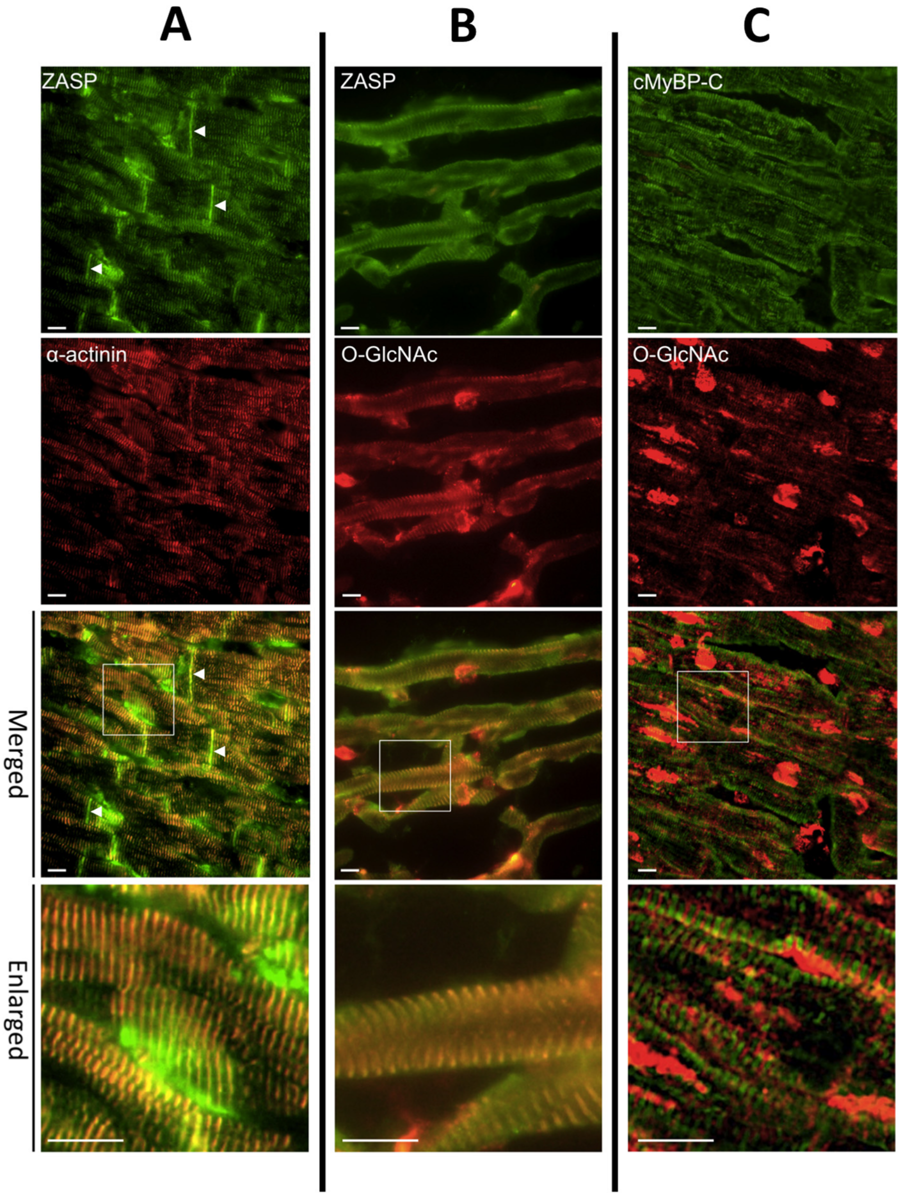

FIGURE 6. **Indirect double immunofluorescence microscopy study showing localization of ZASP and α-actinin (*A*), ZASP and *O*-GlcNAc (*B*), and MyBP-C and *O*-GlcNAc (*C*).** Cryosection thickness = 5 μm. *Scale bars* = 10 μm. *White arrowheads* indicate intercalated discs.

antibody detected seven bands, of which the ZASP1, -2, -3, and -5 bands were indicated to be *O*-GlcNAcylated by the RL2 antibody (Fig. 4*a*). α-Actinin was excluded because the α-actinin antibody consistently detected a band with higher molecular mass than any *O*-GlcNAc or ZASP bands. The possibility of the bands assigned to ZASP being ENH was eliminated by Western blotting with antibody to ENH (Fig. 4*b*).

Human cardiac tissue whole lysate gave a similar profile to the human myofibrils when Western blotted with the RL2 anti-*O*-GlcNAc antibody with 69% of labeling in ZASP bands 1, 2, 3, and 5. However, mouse myofibrils were different from human myofibrils with only 24% of *O*-GlcNAc detected by RL2 in ZASP bands (Fig. 5).

Co-localization of O-GlcNAcylation with ZASP at the Z-disc—Immunofluorescence microscopy showed that ZASP is present in the Z-disc, co-localizing with α-actinin, and also in the intercalated disc (Fig. 6, *column A*). When *O*-GlcNAc antibody RL2 was used, it showed the presence of *O*-GlcNAc-modified proteins in the Z-disc, co-localized with ZASP, and also present in the nucleus (Fig. 6, *column B*). Double immunofluorescence probing with *O*-GlcNAc antibody and cMyBP-C antibody showed that *O*-GlcNAc modification is not located in the C-zone, where the cMyBP-C is located (Fig. 6, *column C*).

Differences in ZASP and O-GlcNAc Levels between Healthy and Diseased Samples—Both the enzymatic labeling and the RL2 antibody showed that the fraction of *O*-GlcNAc labeling of the ZASP bands was greater in failing heart and myectomy muscle samples (Table 1). This could be due to increased *O*-GlcNAc labeling of the ZASP or an increased content of ZASP in the Z-disc.

The amount of ZASP2 (probed with antibody to ZASP) relative to α-actinin (probed with EA-53 antibody to α-actinin) present in human myofibrils was measured and compared between samples. From the ZASP2 band/α-actinin band ratio, we estimated that the amount of ZASP increased in failing and myectomy samples by ~2-fold, whereas there is no trend for the level of ZASP *O*-GlcNAcylation (Fig. 7).

The natural abundance of ZASP in myofibrils was low and not detectable with Coomassie Blue stain when ~20 μg of myofibril protein was separated on a mini SDS-PAGE gel. Its estimated abundance is less than 1 per 400 α-actinin (data not shown). Thus it is likely that ZASP is highly substituted by *O*-GlcNAc.

DISCUSSION

Double immunofluorescence of frozen human cardiac tissues demonstrated that the Z-disc is the most *O*-GlcNAcylated compartment of the sarcomere. Specific enzymatic labeling and probing with two different antibodies to *O*-GlcNAc showed a 90-kDa protein that we identified as ZASP (*LDB3* gene) as the most *O*-GlcNAcylated human myofibril protein. Significantly, *O*-GlcNAc transferase, the enzyme that attaches the *O*-GlcNAc molecule to protein, was also found to localize at the Z-disc (19). Because the quantity of ZASP in the Z-line is estimated to be in the region of 1 per 400 α-actinin molecules, we predict that ZASP is highly modified with *O*-GlcNAc.

It is remarkable that published studies of *O*-GlcNAc modifications in heart muscle, based on measurements with rat or mouse heart, generally show a large number of bands modified by *O*-GlcNAc with only a small proportion of *O*-GlcNAc in bands with apparent molecular mass in the region of 90 kDa (3, 7, 8, 15, 20) and that ZASP has not previously been identified as a highly *O*-GlcNAc-modified protein (4, 21, 22). There are at least three reasons for this. Firstly, the widely used CTD110.6 antibody is not wholly specific to *O*-GlcNAc-substituted proteins. CTD110.6 was described to also label *N*-GlcNAc-modified proteins (23); however, the RL2 antibody and enzymatic labeling methods used here are more specific, and in our study, the 90-kDa band was identified by all three methods. Secondly, due to its low abundance, ZASP may have been excluded in some types of assays, and thirdly, we have shown that the 90-kDa *O*-GlcNAc band is much less prominent in mouse heart when compared with human heart (Fig. 5).

TABLE 1

ZASP *O*-GlcNAcylation (sum of ZASP1, -2, -3, -4, and -5 as identified in Fig. 4) expressed as a percentage of total *O*-GlcNAcylation in donor, failing, and myectomy samples

O-GlcNAcylated band volume was measured by densitometry of SDS-PAGE gels probed by enzymatic labeling with TAMRA, as shown in Figs. 1 and 2, or Western blotting with RL2 antibody, as shown in Figs. 2 and 4. The numbers are the values of the volume of the ZASP bands as a percentage of the volume of all bands in the same lane averaged for 4–10 lanes.

	ZASP, % of total O-GlcNAc		
	Donor	Failing	Myectomy
Enzymic labeling with TAMRA	49 ± 5 (n = 10)	68 ± 9 (n = 4)	76 ± 6[a] (n = 5)
RL2 antibody	80 ± 1 (n = 5)	83 ± 2 (n = 4)	92 ± 7[a] (n = 4)

[a] Significant difference ($p < 0.05$, Student's *t* test) between *O*-GlcNAc percentage in donor and myectomy samples.

The identity of the 90-kDa band as ZASP (also known as Cypher in mouse, and LIM domain-binding protein, LDB3) was established unambiguously by two independent MALDI-MS experiments and by means of ZASP-specific antibodies. In addition, α-actinin and ENH (PDLIM5), both Z-disc-associated proteins of similar mass to ZASP, were excluded using specific antibodies. α-Actinin is larger than ZASP, and ENH is smaller (Fig. 4). When probed with its specific antibody, seven ZASP bands were observed in human myofibrils including three at ~90 kDa that were detected as the main *O*-GlcNAcylated bands by RL2 antibody. With RL2, the 90-kDa band (ZASP2) was the most *O*-GlcNAcylated in all human myofibril and whole lysate samples, and ZASP2 was also the only band consistently detected by enzymatic labeling with TAMRA. The ZASP homologue in mouse, Cypher, is expressed as six isoforms: four long isoforms (723, 679, 661, 622 amino acids) and two short forms (327 and 288 amino acids) that lack the C-terminal LIM domains (24, 25). The observed multiple ZASP bands may correspond to these isoforms but could also be due to protein cleavage.

ZASP is classified as a member of the enigma family, with a PDZ domain near its N terminus and three LIM domains near the C terminus (24, 26) (see supplemental Table 3). It is one of the proteins found in the Z-disc of the sarcomere in both skeletal and cardiac muscles, and binds to other Z-disc proteins such as α-actinin (27), calsarcin, and myotilin (28).

ZASP plays an important, but undefined role in development and maintenance of the myofibril. Cypher knock-out mice have a lethal phenotype (29), and mutations in the *LDB3* gene are associated with cardiomyopathies including dilated cardiomyopathy and left ventricular noncompaction (30–33). ZASP binds to α-actinin via its N-terminal PDZ domain and to other Z-disc proteins to maintain Z-disc structure. It possibly plays a

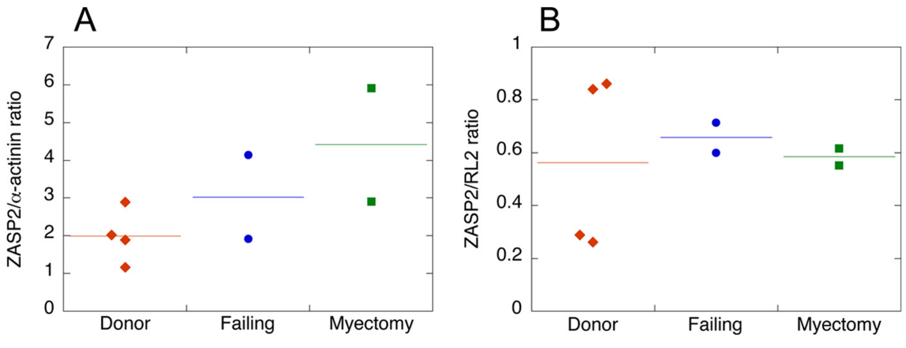

FIGURE 7. *A*, dot-plots showing ratios of ECL signals from Western blots of SDS-PAGE separation of donor myofibrils probed with anti-ZASP (ZASP2 band) and α-actinin antibody. Increased ratio indicates increased ZASP in the myofibrils. *B*, dot-plots showing ratios of ECL signals from Western blots of SDS-PAGE separation of donor myofibrils probed with anti-ZASP (ZASP2 band) and anti-*O*-GlcNAc (RL2 antibody). Increased ratio indicates decreased *O*-GlcNAc content of ZASP.

signaling role through its C-terminal LIM domains binding to PKC (10) and is a potential mechanotransducer, in concert with other Z-disc proteins, which respond to mechanosensation (34, 35). The LIM domains are only present in the long ZASP isoforms and may be the site of *O*-GlcNAcylation. Although we do not know yet whether ZASP is phosphorylated by PKC in physiological conditions, it is interesting to note, for further investigation, the often observed yin-yang relationship between phosphorylation and *O*-GlcNAcylation (2).

Changes in protein *O*-GlcNAcylation have been associated with pathological conditions in the heart. Increases in global *O*-GlcNAcylation were reported in human aortic stenosis and in diabetes, myocardial infarction, and hypertension in rats (3, 19, 20, 36, 37). We observed a corresponding increase in the proportion of total *O*-GlcNAcylation incorporated into ZASP in end stage failing heart and in myectomy samples from patients with hypertrophic obstructive cardiomyopathy (Table 1). However, this may be a consequence of recruitment of ZASP to the Z-disc rather than an increased level of *O*-GlcNAcylation.

Because ZASP has both a structural and a mechanotransducing role in the Z-disc, our finding that it is modified by the potential signaling adduct *O*-GlcNAc raises many interesting and important questions to be answered in future studies.

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