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Interpretation of cardiac troponin T behaviour in size-exclusion chromatography

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Abstract

Background: Knowledge about the presence of intact cardiac troponin T (cTnT) and/or its immunoreactive fragments is of great value for the interpretation of cTnT clearance from the circulation. Until now there has been a lot of controversy about cTnT fragmentation. To provide an answer to this controversy, we investigated fragmentation of cTnT with size-exclusion chromatography (SEC), and confirmed our data using mass spectrometry.
Methods: A highly purified human cTnT standard, characterised using mass spectrometry as a single peak of 34,377 Da and using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) as a single immunoreactive band (37 kDa), was incubated in serum for 0, 24 and 48 h at 37°C and analysed using SEC. A troponin TIC complex standard, used in an earlier study, was also investigated.
Results: We demonstrated that, because of its rod-like shape, the molecular weight of cTnT cannot be estimated from SEC using the molecular weight of globular proteins as a reference. The Stokes radius of intact cTnT was calculated to be 33.7 Å. Incubation of both cardiac troponin standards in troponin-free serum resulted in a time-dependent decrease in intact cTnT and a simultaneous increase in smaller immunoreactive fragments (13.4 and 22.4 Å).
Conclusions: cTnT has a Stokes radius of 33.7 Å. Compared with globular calibrator proteins, intact cTnT elutes earlier than expected based solely on its molecular weight. For non-globular or uncharacterised proteins, Stokes radii should be used for correct interpretation of SEC data. By doing so, we were able to clearly demonstrate cTnT fragments.
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Keywords: cardiac troponin T; mass spectrometry; SDS-PAGE; size-exclusion chromatography; Stokes radius.

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Introduction

Several studies have discussed the presence of circulating cardiac troponin T (cTnT) fragments in patients with acute myocardial infarction (AMI). The latest contribution to the discussion has been by Fahie-Wilson et al. (1), who investigated the nature of cTnT reactive molecules in serum samples from dialysis patients and patients with acute coronary syndrome using gel-filtration or size-exclusion chromatography (SEC). The authors claimed that circulating cTnT in patients with kidney failure and elevated serum cTnT concentrations were due to the presence of the free, intact molecular form of cTnT (1). They also showed that the cTnT elution profiles for patients who had experienced an acute coronary event were identical to those for patients with kidney failure, in contrast to the study by Wu et al., who reported the presence of immunoreactive cTnT fragments in serum after AMI using the same technique (2).

Troponin T fragments were described in the early days of troponin investigation (3, 4). Although these experiments focused mainly on bovine cardiac troponins, it is reasonable to assume that similar results would be obtained for human troponins. Comparison of the amino acid sequences of human and bovine cardiac troponins in the online SWISS-PROT database indicated 85%, 89% and 99% homology between bovine and human cardiac troponin T, I and C, respectively (5). Similar homology has been found for cTnT by others (6). More recently, Labugger et al. also demonstrated cTnT fragments in serum after AMI using a Western blot-direct serum analysis protocol. Importantly, they showed a time-related change in the composition of the fragments (7). Later, Diris et al. (8) and Michielsen et al. (9) showed the presence of cTnT fragments in serum from haemodialysis patients.

When investigating proteins with SEC, it is important to realise that cTnT is far from globular. Numerous studies have already addressed the important influence of the hydrodynamic volume and molecular shape of proteins on chromatographic behaviour in a SEC column (10–15). Ideally, the distribution of a molecule in the pores of a SEC column is only a matter of steric interaction or hydrodynamic volume.

The hydrodynamic volume of a protein can be expressed as the Stokes radius (R_s) and is a generally accepted method for data interpretation in SEC (12). A non-globular protein is known to have a (much) larger R_s compared to a globular protein of equal molecular weight. Several mathematical models have been proposed for description of the behaviour of non-globular proteins in SEC (11, 13). Although none of these models is completely correct, there is general agreement that separation of non-globular proteins in

a SEC column is better described using the Stokes radii of the proteins than using their molecular weights (10). In contrast, when using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) for molecular weight determination, it is possible to directly compare molecular weights with known molecular weight standards. With this technique there is a linear relation between the length of the molecule and the amount of SDS bound to it, and chromatographic behaviour is primarily determined by the negative electrical charge imparted by SDS.

Evidently, when using SEC, the molecular weight of a cTnT molecule, which has a rod-like shape (16, 17), as well as the molecular weight of the troponin TIC complex, cannot be determined directly from a molecular weight calibration curve using globular calibration proteins. The intact cTnT molecule is most likely to elute from the SEC column much earlier than expected based on its relatively large Stokes radius. To test this theory for human cTnT, we performed SEC experiments using a Superdex 75 SEC column, SDS-PAGE and immunoblotting, and mass spectrometry (MS). Further characterisation of serum cTnT and/or its fragments was performed using these three techniques.

Materials and methods

To be able to compare our data with previous findings, we not only used a highly purified (>98% pure) human cTnT standard (Advanced Immunochemical Inc., Long Beach, CA, USA), but also purchased the less well-characterised troponin TIC complex standard (SCIPAC, Sittingbourne, UK) used by Fahie-Wilson et al. (1). MS was used to confirm the molecular weight of all troponin standards. For this purpose, a PBS-IIc time-of-flight mass spectrometer (Ciphergen Biosystems Inc, Fremont, CA, USA) was calibrated in the molecular range 12–147 kDa. Troponin standards were applied directly to a matrix-assisted laser desorption/ionisation (MALDI) surface (2 μL). A saturated sinapinic acid (Ciphergen) solution in 50% acetonitrile, 0.5% trifluoracetic acid was used as the energy-absorbing matrix (two 1-μL additions).

In addition, SDS-PAGE and immunoblotting were performed with monoclonal anti-cTnT antibody 9G6 (HyTest, Turku, Finland), recognising amino acid residues 1–60. Troponin standards were directly diluted to the appropriate concentrations in Laemmli sample buffer (Biorad, Veenendaal, The Netherlands) before application to the electrophoresis gel. We applied 20 μL of this mixture to a 4%–15% linear gradient Tris-HCl polyacrylamide precast gel (BioRad). The Precision Plus Protein Standard (BioRad) was used as the molecular mass marker. After stacking for 15 min at 100 V and running for 45 min at 150 V, the gel was blotted onto a 0.45-μm nitrocellulose membrane (BioRad) at 4°C at 100 V for 60 min. The membrane was blocked for 60 min in PBS containing 33 g/L non-fat dry milk (Nutricia, Cuijk, The Netherlands). The primary monoclonal anti-cTnT antibody (9G6) was added at a 1:1000 dilution in wash buffer (PBS containing 0.2 mL/L Tween-20 and 6.7 g/L non-fat dry milk) and incubated overnight at 4°C. The membrane was washed three times for 5 min in wash buffer and the secondary antibody (peroxidase-labelled goat-anti-mouse; DAKO, Glostrup, Denmark) was then added at a 1:5000 dilution in wash buffer and incubated for 60 min at 4°C. The membrane was washed four times for 5 min in wash buffer and finally once in PBS for 10 min. The membrane was developed with Enhanced Chemiluminescence Buffer (Perkin-Elmer, Boston, MA, USA) and captured on a Kodak X-Omat Blue film (Rochester, USA).

SEC experiments were performed on a Hewlett-Packard 1100 system (Agilent Technologies, Amstelveen, The Netherlands) equipped with a Superdex 75 10/300 GL SEC column (GE Healthcare, Uppsala, Sweden) and a diode array detector (210–900 nm). Ammonium bicarbonate (0.1 mol/L) was used as the running buffer at a flow of 0.25 mL/min to facilitate easy transfer to the mass spectrometer. All samples were loaded onto the column using a 200-μL injection loop. Eq. [1] was used to translate elution volumes into dimensionless theoretical fractional volumes:

$$K_{av} = \frac{V_e - V_0}{V_t - V_0}, \quad [1]$$

where K_{av} is the theoretical fractional volume of the column to which the sample of interest has access, V_e is the elution volume, V_0 is the void volume of the column (7.55 mL) and V_t is the total volume of the column (25 mL).

The low-molecular-weight calibration standard (Pharmacia Biotech, Diegem, Belgium; cat. no. 17-0442-01) was composed of a mixture of four globular proteins: bovine serum albumin (67 kDa, 35.5 Å), ovalbumin (43 kDa, 30.5 Å), chymotrypsinogen A (25 kDa, 20.9 Å) and ribonuclease A (14 kDa, 16.4 Å) (18). Dextran blue (2000 kDa) was used to determine V_0 of the column. Between 20 and 100 min, fractions of 0.5 mL were collected every 2 min. cTnT concentrations were measured on an Elecsys 2010 analyser (Roche Diagnostics, Almere, The Netherlands) with the third-generation cTnT assay, which has a serum detection limit of <0.010 μg/L and between-day variation of 7.9% and 6.7% at concentrations of 0.134 and 2.85 μg/L, respectively (n=89). cTnI was measured with the Access AccuTnI assay (Beckman Coulter Inc., Fullerton, CA, USA). Between-day variation is 3.0% and 4.1% at concentrations of 0.56 and 7.31 μg/L, respectively (n=120).

Pooled serum, obtained from healthy volunteers and negative for both cTnT and cTnI, was spiked with the purified cTnT standard and incubated for 0, 24 and 48 h at 37°C. The negative control was also incubated for 48 h. All samples were separated on the SEC column to investigate possible degradation due to protease activity. Identical experiments were performed using the troponin TIC complex standard, although incubation times were slightly different (0, 24 and 96 h).

Results

Elution profile of globular calibration standards

Figure 1A shows the elution profile of the four globular calibration proteins. The volume at the peak maximum was used for calculation of K_{av} and construction of the calibration curve. K_{av} was plotted against $\ln(R_s)$ to generate a calibration curve for these experimental settings (Figure 1B). With the calibration curve

$$K_{av} = -0.278 \ln(R_s) + 1.077 \quad [2]$$

it is possible to calculate Stokes radii of uncharacterised proteins. For this Superdex 75 column, the R_s separation window (K_{av} between 0 and 1) lies between 1.3 and 48 Å. Any protein with R_s greater than

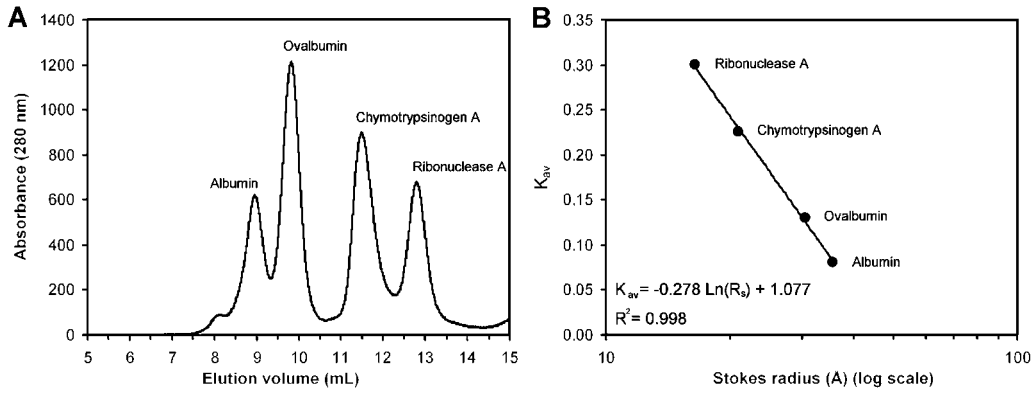

Figure 1 Calibration of the Superdex 75 column. (A) Elution profile of four globular proteins. The theoretical fractional volume (K_{av}) was calculated from Eq. [1]. The void volume (V_0) was determined by elution of dextran blue. The total column volume (V_t) was obtained from the specifications provided by the manufacturer (V_t=25 mL). (B) Calibration equation used for calculation of R_s for uncharacterised proteins.

48 Å will elute at V_0 (7.55 mL) and any protein smaller than 1.3 Å at V_t (25 mL).

Properties of the cardiac troponin standards

Figure 2A,B shows mass spectra of the two cardiac troponin standards. The purified cTnT standard showed one peak at m/z 34,378, corresponding to the molecular weight of the single protonated intact protein ($[M+H]^+$). Also visible was a smaller peak at approximately half this value (m/z 17,307), representing the double protonated cTnT molecule $[M+2H]^{2+}$. In addition, there was also a peak at m/z value 68,545, corresponding to $[2M+H]^+$. In contrast, the major component of the troponin TIC complex standard was albumin (m/z 66,318, $[M+H]^+$). Again, a smaller peak at m/z value 33,238 represents the double protonated albumin molecule $[M+2H]^{2+}$. This mass spectrum did not show peaks at the expected m/z values for the individual troponin T, I or C subunits, or for the TIC complex. It is questionable whether cTnT can be detected at all, due to the relatively high concentration of albumin (5.6 g/L), which is known to cause ion suppression.

Figure 2C shows both standards spiked into serum and applied directly to SEC. The SCIPAC TIC complex

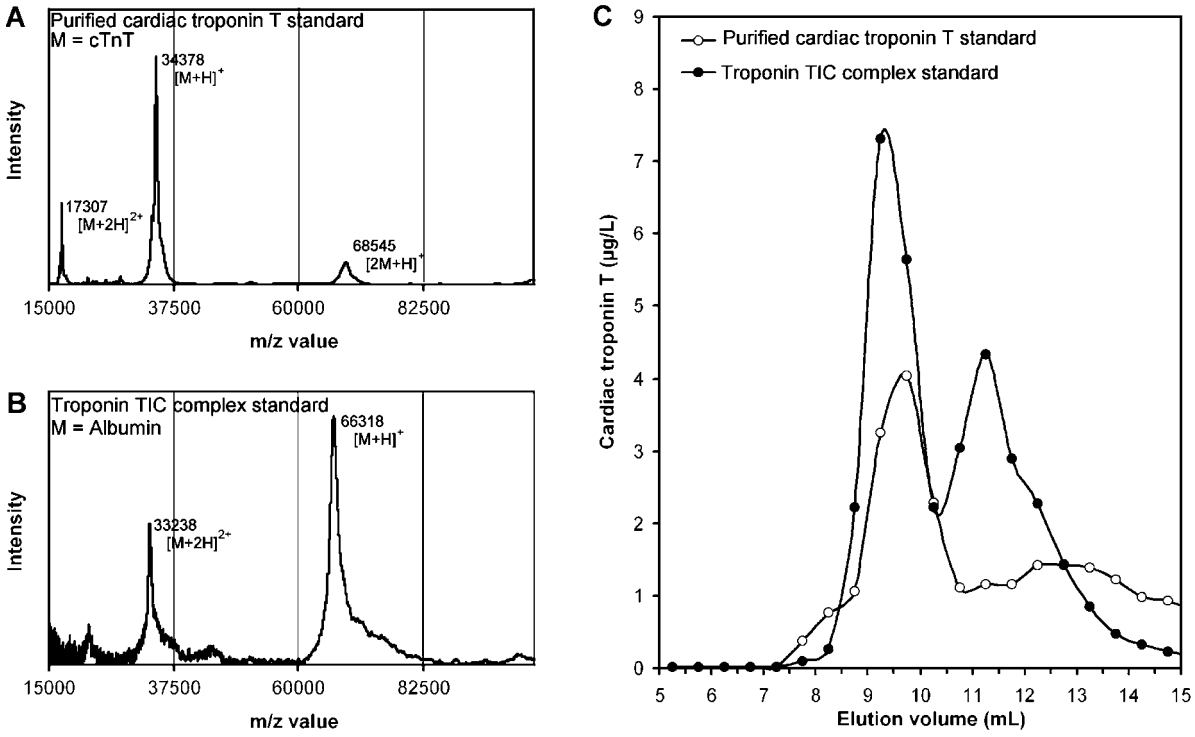

Figure 2 Characterisation of two cardiac troponin standards. (A) Mass spectrum of a purified cTnT standard showing intact protonated cTnT at m/z 34,378 ($[M+H]^+$). (B) Mass spectrum of a troponin TIC complex standard showing albumin at m/z 66,318 ($[M+H]^+$) as the major component. (C) Elution profile of both cardiac troponin standards spiked into serum and applied directly to SEC. cTnT was measured with the third-generation Roche cTnT immunoassay.

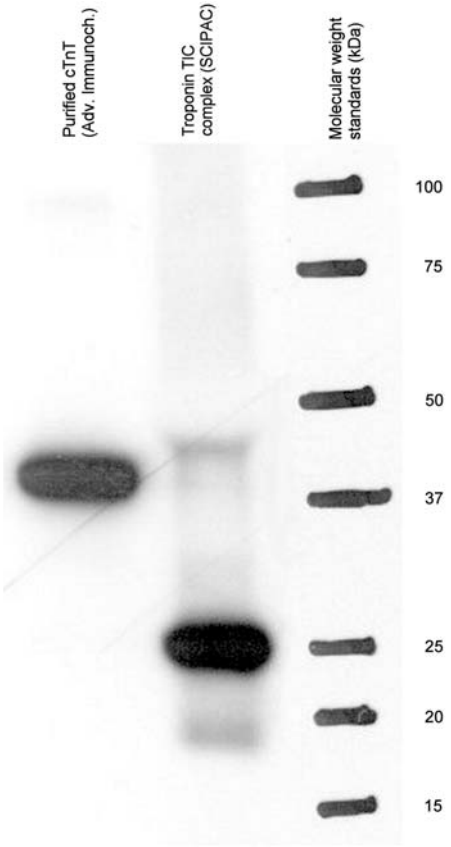

Figure 3 Western blot of both cardiac troponin standards applied directly to SDS-PAGE. Detection was carried out with monoclonal anti-cTnT antibody 9G6 (epitope 1–60, HyTest). The intact protein is partly masked by the large amount of albumin present in the troponin TIC complex standard.

standard showed two cTnT reactive peaks, whereas the purified standard showed one peak superimposed on a column artefact (baseline elevation).

SDS-PAGE confirmed the presence of the intact protein at 37 kDa in the purified cTnT standard. Although partly obscured by the large amount of albumin, the troponin TIC complex standard also showed the intact protein. However, a cTnT fragment at ~27 kDa was most prominent (Figure 3). Albumin removal (ProteoExtract Albumin Removal Kit, Calbiochem, San Diego, CA, USA) did not yield better results (data not shown).

In vitro incubation of troponin standards in serum

Figure 4A, B shows elution profiles for the purified cTnT standard and the troponin TIC complex standard, respectively. Both cardiac troponin standards were spiked into pooled troponin-negative serum and incubated for 0, 24 and 48 or 96 h at 37°C. The x-axis (elution volume) and y-axis (cTnT concentration) are similar for both samples for easier visual comparison. Figure 4 A,B shows two major peaks. Over time, the amount of troponin T eluting in peak 1 (K_{av}=0.097, R_s=33.7 Å) decreased, whereas the amount eluting in peak 2 (K_{av}=0.212, R_s=22.4 Å) increased. Note that peak 2, because of its later appearance, represents a molecule that is smaller than the first peak, which has a molecular weight of 34,377 Da as confirmed by MS. After 96 h of incubation, the troponin TIC complex standard showed a third peak (peak 3, K_{av}=0.355, R_s=13.4 Å), indicating an even smaller immunoreactive cTnT molecule. A summary of these data is given in Table 1.

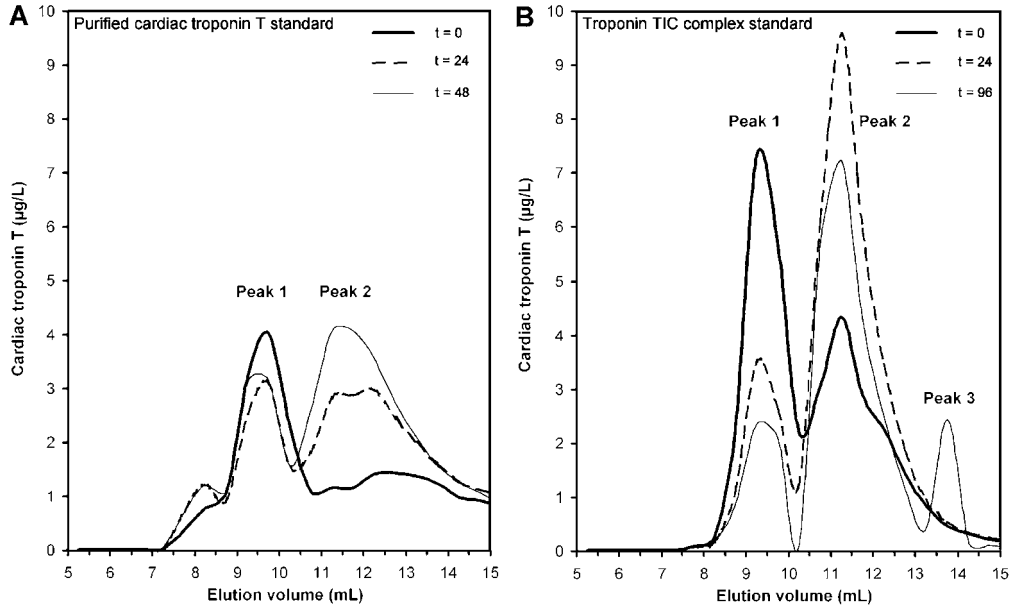

Figure 4 Incubation of cardiac troponin standards in serum. (A) Elution profile of the purified cTnT standard incubated in serum for 0, 24 and 48 h. (B) Elution profile of the troponin TIC complex standard incubated in serum for 0, 24 and 96 h. Both cardiac troponin standards showed a decrease in cTnT concentration for peak 1 and an increase for peak 2. The troponin TIC complex standard incubated for 96 h showed a third immunoreactive cTnT peak. cTnT was measured with the third-generation Roche cTnT immunoassay.

Table 1 Summary of chromatographic data.

	Molecular weight, kDa	V_e, mL	K_{av}	Stokes radius, Å
Calibration proteins				
Ribonuclease A	13.7	12.79	0.301	16.4
Chymotrypsinogen A	25.0	11.49	0.226	20.9
Ovalbumin	43.0	9.82	0.130	30.5
Bovine serum albumin	67.0	8.96	0.081	35.5
cTnT reactive peaks				
Peak 1	34[a]	9.25	0.097	33.7[b]
Peak 2		11.25	0.212	22.4[b]
Peak 3		13.75	0.355	13.4[b]

[a] Molecular weight as confirmed a priori by mass spectrometry. [b] Stokes radius calculated from Eq. [2].

Discussion

When using SEC for molecular weight determination of uncharacterised proteins, it is important to realise the molecular mechanism behind the technique. Although the ideal situation can be described quite precisely, laboratory practice has proven to be different. When trying to label a peak with a certain molecular weight, thorough knowledge about the sample, as well as calibrators and standards, is of utmost importance.

Regarding our data, the purified cTnT standard showed the presence of only one protein with a molecular weight of 34,377 Da, as confirmed by MS and similar to the expected mass of intact cTnT (Figure 2A). In contrast, the troponin TIC complex standard showed no peak at the expected molecular weight of the troponin TIC complex (77 kDa), nor at the expected position of individual subunits T, C and I. This might be attributable to (a) the effects of the MALDI technique, which can cause separation of the TIC complex into the three individual troponin subunits, combined with (b) the relatively high (5.6 g/L) albumin concentration, resulting in suppression of the troponin signals. Therefore, from the absence of a peak at m/z ~77,000 in Figure 2B it may not be concluded that no troponin TIC complex is present. In addition, under denaturing conditions, as with SDS-PAGE, protein complexes are likely to be separated into individual subunits. This might explain why the native TIC complex was not detected in our experiments.

For the bovine cardiac troponin TIC complex, R_s of 52–57 Å has been found (4, 19). The online SWISS-PROT database indicated 85%, 89% and 99% homology between bovine and human cardiac troponin T (6), I and C, respectively (5). In our opinion, this justifies application of these bovine data to human troponins. The properties of the Superdex 75 column limit the highest Stokes radius that can be determined to 48 Å. Therefore, the intact troponin TIC complex should, if present, elute at V_0 ($K_{av}=0$).

Separation of the cardiac troponin standards should result in either one peak for the purified cTnT standard (intact cTnT), and one, or maybe two peaks for the troponin TIC complex standard (troponin TIC complex, and intact cTnT). Bearing in mind the non-globular shape of cTnT, the actual elution volumes for both intact cTnT and the troponin TIC complex would be shifted towards larger elution volumes. Importantly, the peak for the troponin TIC complex would elute earlier than the peak for intact cTnT. Surprisingly, when both cardiac troponin standards were loaded onto the column, both showed a similar elution profile (Figure 2C), revealing two major peaks. In combination with the data derived from the MS experiments with the purified cTnT standard, this leads to the following conclusions. (a) In the absence of troponin I and C, peak 1 for the purified cTnT standard (the content of which was confirmed by MS) can only be intact cTnT, and not the TIC complex. (b) R_s of peak 1 (33.7 Å) is too small for the earlier reported R_s of 52–57 Å for the troponin TIC complex (4, 19), thus supporting the first conclusion. (c) Following conclusion (a), peak 2 must be an immunoreactive fragment of cTnT, most likely the ~25-kDa fragment described by our group (9) and others (7, 20). (d) Based on identical elution volumes, the two peaks of the troponin TIC complex standard can only represent intact cTnT and a smaller immunoreactive fragment. (e) Under the current chromatographic conditions, the TIC complex is unstable and disintegrates into its subunits. (f) R_s of intact human cTnT is 33.7 Å.

In vitro incubation of both cardiac troponin standards in serum showed a time-dependent decrease in peak 1 and a concomitant increase in peak 2. For the troponin TIC complex standard, a third, even smaller fragment appeared at t=96 h. The small cTnT reactive peak, only present after 24 or 48 h of incubation of the highly purified cTnT standard (Figure 4A), suggests the presence of a molecule with higher R_s than intact cTnT. Possible cTnT aggregation (16) does not interfere with the R_s measured for this fraction (which is only slightly greater than the R_s of the monomer) when it is assumed that the two rod-like molecules adhere along the long axis. Evidently, it cannot be the troponin TIC complex due to the absence of cTnI and the fact that it would elute at V_0.

Based on the previous conclusions, this degradation of cTnT confirms earlier experiments in which in vitro incubation of spiked serum samples showed the appearance of smaller degradation products in time on SDS-PAGE [Figure 1 in the online data supplement of Michielsen et al. (9)]. The latest article on cTnT fragmentation by Fahie-Wilson et al. (1) explicitly claimed that cTnT circulates in the free, intact form in patients with kidney failure. However, they did not take into

account the rod-like shape of cTnT. We calculated, based on their data (extracted from the text and figures), comparable Stokes radii (37 Å for peak 1 and 26 Å for peak 2) for the two major cTnT reactive peaks (21). The specifications of their SEC column limit the separation range from 5 to 48 Å, so if the large troponin TIC complex (R_s = 52–57 Å) were present, it would have eluted at V_0. However, no cTnT reactive peak was demonstrated at V_0.

In conclusion, based on the comparable Stokes radii found in our study compared with the results described by Fahie-Wilson et al. (1) and the independent confirmation of the molecular mass of the purified cTnT standard by MS and SDS-PAGE, we may conclude that the peaks indicated by Fahie-Wilson et al. (1) cannot be the troponin TIC complex and free cTnT, but are intact cTnT and an immunoreactive cTnT degradation product (most probably of approx. 25 kDa). Hence, the correct conclusion from the study by Fahie-Wilson et al. (1) should have been that in patients on haemodialysis or peritoneal dialysis, no intact free cTnT is present, but a smaller (25 kDa) immunoreactive cTnT fragment is. In patients with acute coronary syndrome, a small amount of the intact free protein is present, depending on the time after onset of symptoms. Moreover, this conclusion is strengthened by the fact that incubation of a purified cTnT standard in troponin-free serum resulted in a time-dependent increase in immunoreactive fragments, which has been demonstrated in this study by SEC chromatography and in an earlier study by our group using SDS-PAGE (9).

With our current findings, we clearly demonstrate that a thorough approach to interpretation of SEC data is of utmost importance and that fragmentation of cTnT in serum is clearly demonstrated using different techniques.

Limitations of the study

Because of the unavailability of unlabelled anti-cTnT antibodies for the Roche third-generation cTnT assay, a different anti-cTnT antibody had to be used. Therefore, it should be noted that (quantitative) data from the immunoblot cannot be directly compared to the peak height for SEC data.

References

1. Fahie-Wilson MN, Carmichael DJ, Delaney MP, Stevens PE, Hall EM, Lamb EJ. Cardiac troponin T circulates in the free, intact form in patients with kidney failure. Clin Chem 2006;52:414–20.
2. Wu AH, Feng YJ, Moore R, Apple FS, McPherson PH, Buechler KF, et al. Characterization of cardiac troponin subunit release into serum after acute myocardial infarction and comparison of assays for troponin T and I. American Association for Clinical Chemistry Subcommittee on cTnI Standardization. Clin Chem 1998;44:1198–208.
3. Burtnick LD, McCubbin WD, Kay CM. The isolation and characterization of the tropomyosin binding component (TN-T) of bovine cardiac troponin. Can J Biochem 1976;54:546–52.
4. Byers DM, McCubbin WD, Kay CM. Hydrodynamic properties of bovine cardiac troponin. FEBS Lett 1979;104: 106–10.
5. Boeckmann B, Bairoch A, Apweiler R, Blatter MC, Estreicher A, Gasteiger E, et al. The SWISS-PROT protein knowledge base and its supplement TrEMBL in 2003. Nucleic Acids Res 2003;31:365–70.
6. Mesnard L, Samson F, Espinasse I, Durand J, Neveux JY, Mercadier JJ. Molecular cloning and developmental expression of human cardiac troponin T. FEBS Lett 1993;328:139–44.
7. Labugger R, Organ L, Collier C, Atar D, Van Eyk JE. Extensive troponin I and T modification detected in serum from patients with acute myocardial infarction. Circulation 2000;102:1221–6.
8. Diris JH, Hackeng CM, Kooman JP, Pinto YM, Hermens WT, Van Dieijen-Visser MP. Impaired renal clearance explains elevated troponin T fragments in hemodialysis patients. Circulation 2004;109:23–5.
9. Michielsen EC, Diris JH, Hackeng CM, Wodzig WK, Van Dieijen-Visser MP. Highly sensitive immunoprecipitation method for extracting and concentrating low-abundance proteins from human serum. Clin Chem 2005;51:222–4.
10. Cabre F, Canela EI, Canela MA. Accuracy and precision in the determination of Stokes radii and molecular masses of proteins by gel filtration chromatography. J Chromatogr 1989;472:347–56.
11. Potschka M. Universal calibration of gel permeation chromatography and determination of molecular shape in solution. Anal Biochem 1987;162:47–64.
12. Himmel ME, Baker JO, Mitchell DJ. Size exclusion chromatography of proteins. In: Wu C-S, editor. Handbook of size exclusion chromatography. New York: Marcel Dekker, 1995:409–28.
13. Dubin PL, Principi JM. Failure of universal calibration for size exclusion chromatography of rodlike macromolecules versus random coils and globular proteins. Macromolecules 1989;22:1891–6.
14. Demassieux S, Lachance J-P. Molecular filtration on Sepharose 6B. Empiric relationships between the distribution coefficient and the Stokes radius on the one hand and the molecular weight on the other of proteins of different degrees of asymmetry. J Chromatogr 1974;89: 251–8.
15. Axelsson I. Characterization of proteins and other macromolecules by agarose gel chromatography. J Chromatogr A 1978;152:21–32.
16. Byers DM, Kay CM. Hydrodynamic properties of bovine cardiac troponin-I and troponin-T. J Biol Chem 1983;258: 2951–4.
17. Flicker PF, Phillips GN Jr, Cohen C. Troponin and its interactions with tropomyosin. An electron microscope study. J Mol Biol 1982;162:495–501.
18. Fasman GD. Handbook of biochemistry and molecular biology. Section A: proteins, 3rd ed. Cleveland, OH: CRC Press, 1976.
19. Hinkle A, Goranson A, Butters CA, Tobacman LS. Roles for the troponin tail domain in thin filament assembly and regulation. A deletional study of cardiac troponin T. J Biol Chem 1999;274:7157–64.
20. Communal C, Sumandea M, de Tombe P, Narula J, Solaro RJ, Hajjar RJ. Functional consequences of caspase activation in cardiac myocytes. Proc Natl Acad Sci USA 2002;99:6252–6.
21. Michielsen EC, Diris JH, Kleijnen VW, Wodzig KW, van Dieijen-Visser MP. Size-exclusion chromatography of circulating cardiac troponin T [letter]. Clin Chem 2006. In press.

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