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COMPARISON OF *IN VITRO* STABILITY OF RECOMBINANT AND NATIVE HUMAN CARDIAC TROPONIN COMPLEXES

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Introduction

Ternary troponin complex (ITC) is formed by three proteins (troponin I, troponin T, and troponin C (TnC)) and it plays a key role in the regulation of muscle contraction. Specific cardiac isoforms of troponin T (cTnT) and troponin I (cTnI) are widely used as biomarkers of heart injuries.

At present, the international immunochemical standard for cardiac troponins is the native ternary troponin complex (natITC) which, apparently, corresponds to one of the main forms of cardiac troponins in the blood of patients shortly after acute myocardial injuries. ITC, which consists of recombinant proteins, can be a more convenient alternative to the native complex, although it requires more thorough characterization and verification. The aim of this work was to compare the stability of recombinant ITC (recITC) and natITC in different conditions.

Materials and methods

Anti-troponin monoclonal antibodies (MAbs) and human natITC and recITC were provided by Hytest (Finland). Native and recombinant ITC-complexes were incubated in two buffer solutions (Buffer 1: 20 mM Tris-HCl, 150 mM KCl, 7.5% bovine serum albumin (BSA), 5 mM $CaCl_2$, 0.1% NaN_3, pH 7.5; Buffer 2: 20 mM Tris-HCl, 150 mM KCl, 5 mM $CaCl_2$, 0.1% NaN_3, pH 7.5) and in normal human serum or in different types of normal human plasma at +4°C, +25°C and +37°C for up to 48 hours.

Cardiac troponins were measured using various sandwich fluoroimmunoassays specific to troponin complexes or cTnI or cTnT. Conjugates of MAbs with stable Eu^{3+}-chelate were used for detection. The assays used in the experiments are depicted in Fig. 1 (numbers in brackets refer to approximate MAbs epitopes, MAb 20C6 is specific to a conformational epitope formed by cTnI and TnC).

Results

Immunochemical activity and stability of ITC-complexes in different buffers

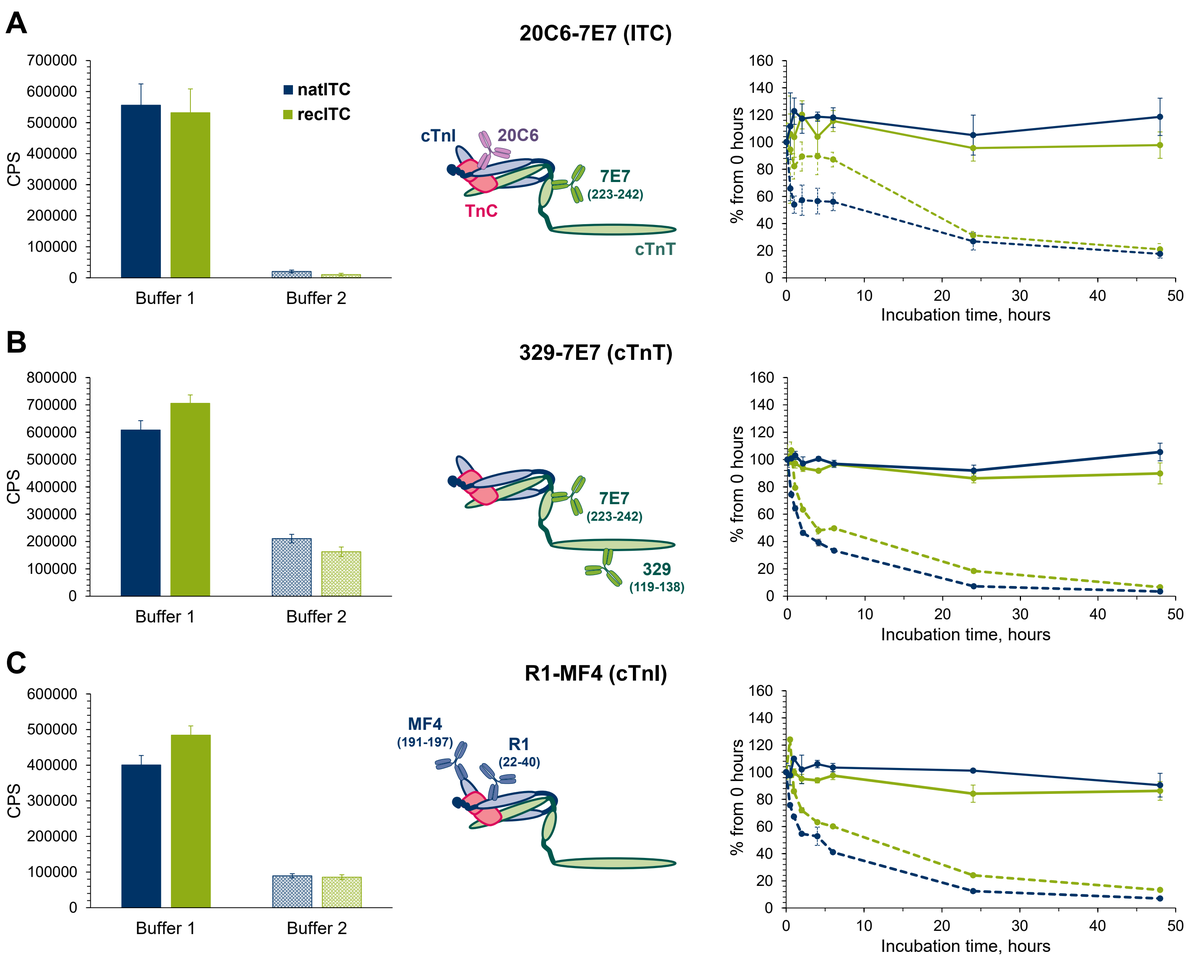

Figure 1. Changes in the immunochemical activity of troponins in ITC at +25°C in different buffers.
A – *ITC;* ***B –*** *cTnT;* ***C –*** *cTnI. Complexes were spiked to a final concentration of 100 ng/mL.*
Left: *ternary troponin complexes (recITC, green, and natITC, blue) were spiked in Buffer 1 (solid filled square), or Buffer 2 (square with dots) without incubation.* ***Right:*** *recITC and natITC were spiked in Buffer 1 (solid line) or Buffer 2 (dashed line) and incubated for up to 48 hours at +25°C. Immunoactivity in the samples without incubation was taken as 100%. Average ± SD.*

Dilution of recITC and natITC in the buffer without BSA led to a significant decrease in their immunochemical activity in comparison with Buffer 1 (Fig. 1). The presence of BSA also preserved the immunochemical activity of both complexes and their individual components during incubation at +25°C for 48 hours.

Immunochemical stability of ITC-complexes at different temperatures

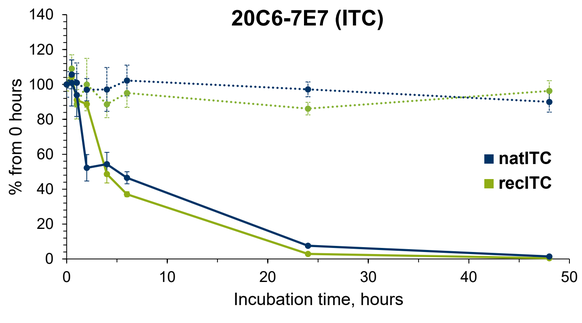

Figure 2. Changes in the immunochemical activity of ITC during incubation at +4°C and +37°C in Buffer 1.
RecITC (green) and natITC (blue) were spiked in Buffer 1 to a final concentration of 100 ng/mL and detected during incubation at +4°C (dotted lines) and +37°C (solid lines) for up to 48 hours. Immunoactivity in the samples without incubation was taken as 100%. Average ± SD.

All of the ternary complexes and individual troponins (data not shown) were stable during incubation at +4°C (Fig. 2). However, the immunochemical activity of both recITC and natITC significantly (>50%) decreased after just 3 hours of incubation at +37°C.

Results

Immunochemical stability of ITC-complexes in different matrices

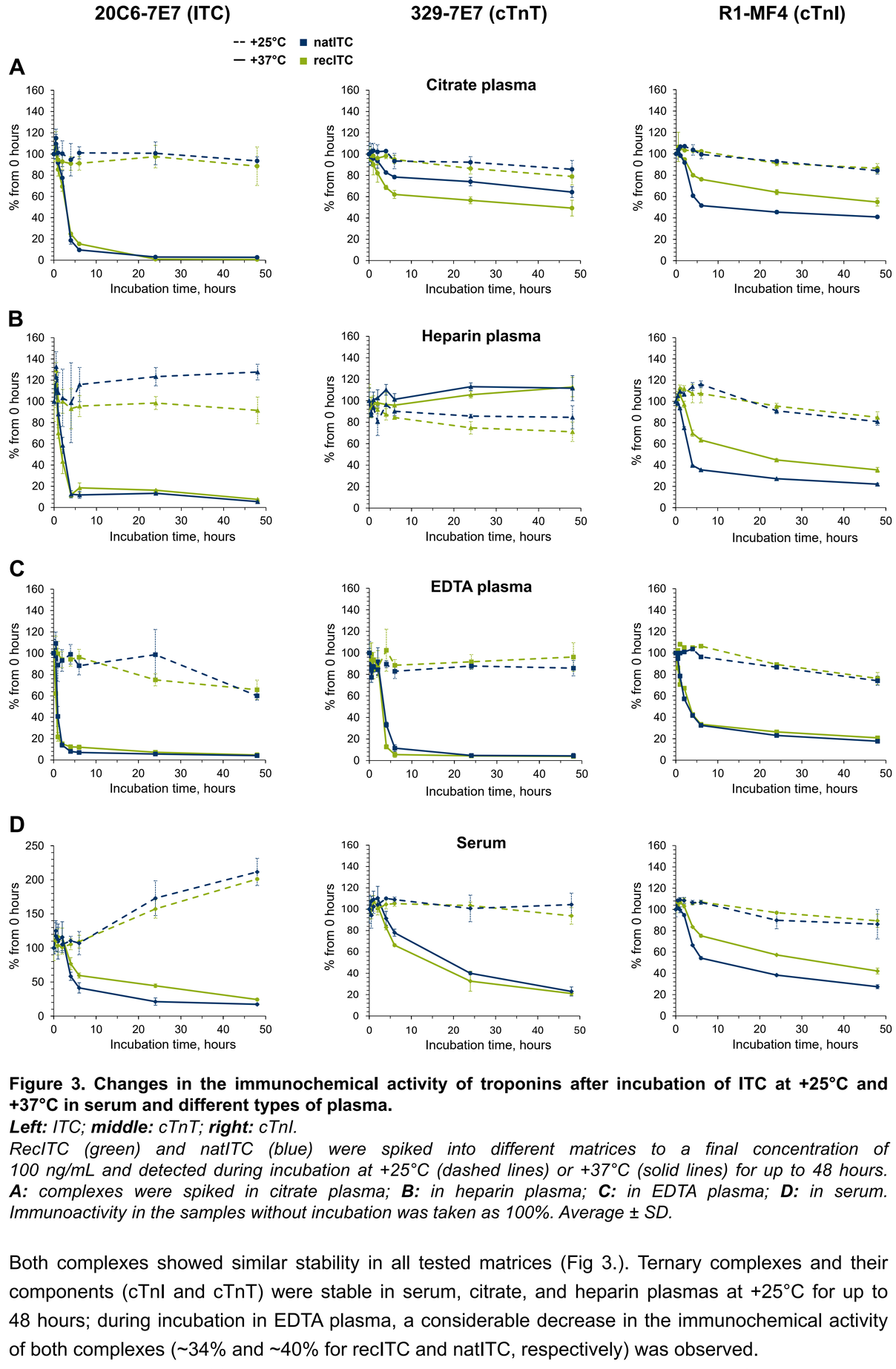

Figure 3. Changes in the immunochemical activity of troponins after incubation of ITC at +25°C and +37°C in serum and different types of plasma.
Left: *ITC;* ***middle:*** *cTnT;* ***right:*** *cTnI.*
RecITC (green) and natITC (blue) were spiked into different matrices to a final concentration of 100 ng/mL and detected during incubation at +25°C (dashed lines) or +37°C (solid lines) for up to 48 hours. ***A:*** *complexes were spiked in citrate plasma;* ***B:*** *in heparin plasma;* ***C:*** *in EDTA plasma;* ***D:*** *in serum. Immunoactivity in the samples without incubation was taken as 100%. Average ± SD.*

Both complexes showed similar stability in all tested matrices (Fig 3.). Ternary complexes and their components (cTnI and cTnT) were stable in serum, citrate, and heparin plasmas at +25°C for up to 48 hours; during incubation in EDTA plasma, a considerable decrease in the immunochemical activity of both complexes (~34% and ~40% for recITC and natITC, respectively) was observed.

When incubated at +37°C, the complexes were most stable in serum, but by the end of incubation, they lost >50% of their immunochemical activity. Both recITC and natITC demonstrated the lowest stability during incubation at +37°C in EDTA plasma. This may be associated with both the dissociation of the complexes and the degradation of individual troponins included in the complexes. The immunochemical activity of both cTnI and cTnT decreased to ~67–68% and ~13–33% after 4 hours of incubation, respectively. In contrast to EDTA plasma, cTnT appeared to be more stable in heparin plasma than cTnI.

Conclusions

The presence of BSA stabilized the immunochemical activity of ITC in the buffer solution.

Both the recombinant and native complexes were stable at +25°C for up to 48 hours in all matrices, except EDTA plasma. At +37°C, dissociation of the complexes occurred in all matrices, and this was accompanied by a considerable decrease in immunochemical activity of cTnI (all matrices) and cTnT (serum, citrate, and EDTA plasmas).

The immunochemical activity and stability of recITC were similar to those of natITC in all of the tested matrices and conditions, which makes it possible to use recITC as an immunochemical standard for the detection of troponins or troponin complexes.