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Chimeric synthetic peptides as antigens for detection of antibodies to *Trypanosoma cruzi*

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Abstract

Six chimeric synthetic peptides (QCha-1, QCha-2, QCha-3, QCha-4, QCha-5, and QCha-6) incorporating antigenic sequences of two immunodominant repeat B-cell epitopes of *Trypanosoma cruzi* were synthesized by conventional solid-phase peptide synthesis. The antigenic activity of these peptides was evaluated by UltramicroEnzyme-linked immunosorbent assay (UMELISA) by using panels of positive Chagasic sera ($n = 82$), while specificity was evaluated with samples from healthy blood donors ($n = 44$) and patients with other infectious diseases ($n = 86$). The antigenicity of the chimeric peptides in solid-phase immunoassays was compared with that of the monomeric peptides. Data demonstrated that the chimeric peptide QCha-5 was the most reactive because it detected antibodies to parasite efficiently. The results indicate that chimeric peptide as coating antigen is very useful for the immunodiagnosis of Chagas' disease.

Keywords: Chimeric synthetic peptides; UMELISA; *Trypanosoma cruzi*; Chagas; Antigenicity

Chagas' disease or *American trypanosomiasis*, caused by the flagellate protozoan *Trypanosoma cruzi* (*T. cruzi*), is one of the most important endemic problems in Americas, particularly in South America [1–4]. Chagas' disease currently affects 16–18 million people. This illness is transmitted to humans and other mammals mostly by insect vectors of the subfamily *Triatominae* [5,6], during transfusion of individuals' blood infected with the protozoan [7], and through other transmission mechanisms: ingestion of food contaminated with parasites, organ transplantation, transmitted through the placenta, mother's milk, laboratory accidents, etc.

The disease affects the nervous system, digestive system, and heart. Chronic infections result in various neurological disorders, including dementia, damage to the heart muscle (cardiomyopathy, the most serious manifestation), and sometimes dilation of the digestive tract (megacolon and megaesophagus), as well as weight loss.

The diagnosis of Chagas' disease is determined by means of the detection of the parasite in the blood samples by direct examination, hemoculture, or xenodiagnosis (direct methods) and/or for the detection of specific antibodies to *T. cruzi* antigens by immunological methods (indirect methods) [8].

Commercial ELISAs use, in solid surface, antigens obtained by epimastigote lysis or trypomastigote of the *T. cruzi* [9,10]. These tests present good sensitivity, but they are inconvenient in that they present cross-reactivity with Leishmania patient sera, for what they obtained frequently false positive results [11,12].

A solution to the problem of the serological diagnosis of the Chagas' disease is the use of recombinant [13] and synthetic peptides [14,15], designed, to obtain a diagnosis test that guarantees results with high levels of sensitivity and specificity. The antigenic sequences that used them more belong to repetitive regions of the parasite [16],

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shed-acute-phase-antigen (SAPA), cytoplasmic repetitive antigen (CRA), trypomastigote surface antigen (TSA), etc.

However, the main disadvantage of the short synthetic peptides is that these are poorly antigenic in solid-phase based immunoassays due to their weak binding to solid surfaces. Also, the synthetic peptides lose their antigenicity because of the masking of essential antigenic side chains following binding to a solid phase. For this reason, there is a tendency to use chimeric synthetic peptides to avoid this problem and to improve the sensitivity and specificity of the assays [17–21].

For this purpose, six chimeric synthetic peptides (QCha-1, QCha-2, QCha-3, QCha-4, QCha-5, and QCha-6) were synthesized. These peptides were designed by means of the combination of two immunodominant repeat B-cell epitopes from *T. cruzi*. The monomeric peptides (P1 and P2) were obtained.

Materials and methods

Solid-phase synthesis. Based on the sequence for *T. cruzi* solid-phase peptide synthesis was performed by using the standard solid-phase method [18–22], as described previously. The monomeric and chimeric peptides (Table 1) were synthesized manually, using Boc chemistry. All solvents used for peptide synthesis were pure. Side-chain-protected amino acids used were from Bachem (Switzerland). As solid phase, the polymer *p*-methylbenzhydryl amine (Bachem, Switzerland) resin was used. The coupling reactions were carried out using DIPCDI (Merck, Germany)/HOBt (Sigma) in DMF. Following, each amino acid coupling one sample was taken and a qualitative ninhydrin assay was performed. After completion of synthesis, peptides were cleaved from the resin and the amino acid side chains were deprotected by acidic hydrolysis using HF pure for analysis (Fluka, USA) in the presence of scavengers (anisole, dimethyl sulfide, 1,2-ethanedithiol, and *p*-cresole) (Merck, Germany).

Table 1
Monomeric and chimeric synthetic peptides that correspond to immunodominant repeat B-cell epitopes from *Trypanosoma cruzi*

Peptides [a]	Sequence
P1	PSPFGQAAAGDK
P2	AEPKPAEPKS
QCha-1	P1-P2
QCha-2	P1-P2-P1
QCha-3	P2-P1-P2
QCha-4	P2-P1
QCha-5	P1-P1-P2
QCha-6	P2-P2-P1

[a] The peptide sequences were deduced from reported by Gruber A et al. [23] and Buschiazzo A et al. [24].

Peptide purification and characterization. The peptides obtained were analyzed by analytical reverse-phase high-performance liquid chromatography (RP-HPLC, Pharmacia, LKB, Sweden), using a RPC18 protein/peptide column (Vydac, 4.6×150 mm) with a linear gradient from solution A (0.1% TFA in water) to solution B (0.05% TFA in acetonitrile), in 35 min. The peptides were detected by UV at $\lambda = 226$ nm. Data were processed by the Biocrom program (CIGB, Cuba).

Peptide molecular weights were verified by electrospray ionization-mass spectrometry (ESI-MS) using mass spectrometer with orthogonal geometry QTOF-2 (Micromass, UK). The spectrum was processed with MassLinx v3.5 (Micromass, UK).

Enzyme-linked immunosorbent assay. UltramicroEnzyme-linked immunosorbent assay (UMELISA) combines the high sensitivity of current ELISA tests with the use of small volumes of samples and reagents. This assay was performed according to that described in [22]. Peptides were dissolved in 0.05 mol/L carbonate–bicarbonate buffer, pH 9.6, and the wells were coated using 15 μL of a solution of the monomeric or chimeric peptides (4 μg/mL).

All assays included positive and negative controls. The fluorescence reading from each sample was normalized as a relative value (FRV) on the fluorescence value of a positive control over the same plate. Samples were considered positive when FRV were equal to or higher than the cutoff value (0.30). All numeric results are means of duplicate.

The analyzed samples were human Chagasic sera ($n = 45$) from Colombia and Brasil ($n = 37$) defined as positive by indirect immunofluorescence assay. Specificity was assessed by using sera from the healthy blood donors ($n = 44$) and 86 subjects with other infectious diseases: human immunodeficiency virus type 1 (HIV-1) ($n = 15$), human immunodeficiency virus type 2 (HIV-2) ($n = 5$), toxoplasmosis ($n = 20$), hepatitis C virus (HCV) ($n = 20$), human T-cell leukemia virus type I (HTLV-I) ($n = 20$), and leprosy ($n = 6$).

Cutoff value: The cutoff value (CO) was 0.30.

Results

Synthesis, purity, and characterization of peptides

Synthesis of our chimeric and monomeric peptides was based on the conventional solid-phase peptide methodology using Boc chemistry. The appropriately protected amino acids were incorporated into the peptide sequence using DIPCDI/HOBt as the coupling reagent. The peptides were obtained in good yield. The purity of the peptides was evaluated by analytical RP-HPLC, and all the peptides were

Table 2
Reactivity of the chimeric peptides, which sequences combine two immunodominant repeat B-cell epitopes from *Trypanosoma cruzi* and the monomeric peptides against the samples of the Chagasic sera from Colombia ($n = 45$) and Brasil ($n = 37$), and 86 subjects with other infectious diseases: human immunodeficiency virus type 1 (HIV-1) ($n = 15$), human immunodeficiency virus type 2 (HIV-2) ($n = 5$), toxoplasmosis ($n = 20$), hepatitis C virus (HCV) ($n = 20$), human T-cell leukemia virus type I (HTLV-I) ($n = 20$), and leprosy ($n = 6$)

Samples	P1	P2	QCha-1	QCha-2	QCha-3	QCha-4	QCha-5	QCha-6
Chagas' sera Colombia ($n = 45$)	31/45	22/45	39/45	38/45	40/45	38/45	45/45	39/45
Chagas' sera Brasil ($n = 37$)	32/37	33/37	34/37	37/37	37/37	36/37	37/37	36/37
HIV-1	0/15	0/15	0/15	0/15	0/15	0/15	0/15	0/15
HIV-2	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
HCV	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20
HTLV-I	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20
Toxoplasmosis	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20
Leprosy	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6

found to be >85% pure. Peptides were successfully characterized by ESI-MS.

Antigenicity

A summary of the antigenicities of the monomeric and chimeric peptides is presented in Table 2. To assess peptide specificity, 86 subjects with other infectious diseases: human immunodeficiency virus type 1 (HIV-1) ($n = 15$), human immunodeficiency virus type 2 (HIV-2) ($n = 5$), toxoplasmosis ($n = 20$), hepatitis C virus (HCV) ($n = 20$), human T-cell leukemia virus type I (HTLV-I) ($n = 20$), and leprosy ($n = 6$) were tested (Table 2). Samples from healthy blood donors were also evaluated ($n = 44$), and all specimens were finally considered negative.

Discussion

Two monomeric peptides (P1 and P2) and six chimeric synthetic peptides (QCha-1, QCha-2, QCha-3, QCha-4, QCha-5, and QCha-6), containing two immunodominant repeat B-cell epitopes, were obtained.

The antigenic activities of the new chimeric and monomeric synthetic peptides were evaluated. All peptides were assessed against the Chagas positive samples from Colombia and Brasil.

The monomeric peptides' performance with Chagas positive samples from Colombia ($n = 45$) and sera from seropositive people from Brasil ($n = 37$) is shown in Table 2, where: peptide P1 detected (31/45) (69%) positive sera, but detected (32/37) (86%) positive samples. Peptide P2 detected (22/45) (49%) positive sera and detected (33/37) (89%) positive samples.

Differences in reactivity to various chimeric synthetic peptides were observed (Table 2). Antibodies against peptides QCha-5 (45/45) (100%) and QCha-3 (40/45) (89%) were found at high levels in most serum samples from Colombia and peptides QCha-2 (37/37) (100%), QCha-3 (37/37) (100%), QCha-4 (36/37) (97%), QCha-5 (37/37) (100%), and QCha-6 (36/37) (97%) with positive sera from Brasil.

These results showed that the chimeric peptides are more antigenic than the monomeric peptides and these can be used to detect antibodies to more than one epitope simultaneously. Our results also showed that the order of location of the epitopes in the chimeric peptides is determinant in the antigenicity of these biomolecules. The P1-P1-P2 epitope orientation was found to be most suitable for an increased interaction with antibodies.

Similar results, regarding the order of location of the epitopes in the chimeric peptides, were reported by us in previous studies when the chimeric peptides of HTLV were evaluated [18–21]. This phenomenon should take place due to the space conformation of the molecule allowing an appropriate exposure to the antibodies.

In conclusion, we showed here that the chimeric peptide QCha-5, incorporating two immunodominant repeat B-cell epitopes of the *T. cruzi*, of this study was the most antigenic peptide. Therefore, this peptide will be useful as antigen for the detection of antibodies to *T. cruzi* and for the control of disease transmission by blood transfusion.

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