SUPPORTING INFORMATION

Physicochemical characterization of the ELR and m-ELRGlu15

The **chemical structures** of functionalized polymer samples were confirmed by ^{1}H NMR, ^{13}C NMR and FT-IR spectroscopy and MALDI-TOF mass spectrometry

Although the peaks in the ^{1}H NMR spectra of the polymers were broad and not very informative, integration of the protons in the aromatic and aliphatic regions allowed us to confirm the respective coupling reaction.

The multiplet at 2.25-2.15 ppm (g) assigned to the methylene protons adjacent to the γ-carboxylic group of glutamic acid was less intense for m-ELR than for the original ELR as the -CH_2- proton resonances shifted downfield from 2.34-2.14 to 2.14-1.61 ppm, as expected. The integration ratios of the aromatic and NH protons (m, 8.55-7.43) increased for azo-NH_2 and FB-NH_2 substrates, and a new band (m, 3.51-3.45) corresponding to the methylene protons, along with other new resonances at the expected positions, appeared for the PEG-NH_2 substrate, thus confirming ELR functionalization and the structure of m-ELRGlu15.

The modification ratio for Azo-Glu15 and FB-Glu15 was readily calculated from the ^{1}H NMR spectra by comparing the integration ratios of the N-H region for the initial ELR with that found for m-ELR, which includes the aromatic C-H's from the azobenzene or phenylboronic groups. Finally, the PEG-Glu15 coupling ratio was calculated from the ratio of the integration areas for the backbone methylene hydrogens in the PEG substrate [3.48 (m)] with the main chain methyl protons (CH(CH_3)$_2$) of valine residues. The conversions obtained were similar to those obtained from absorbance measurements or from the corresponding MALDI-ToF.

The ^{13}C NMR spectra of the m-ELRGlu15s also supporting a structure in which Azo-NH_2, Sp-OH or FB-NH_2 aryl groups, or PEG-NH_2 moieties, are attached to the γ-carboxylic group of the glutamic acid residues found in the original biopolymer.

The carbonyl carbon of the glutamic residues (171.84 ppm) was replaced by the corresponding carbonyl from the amide/ester bond in m-ELRGlu15 (171.7, 171.54, 171.58 and 171.58 ppm) and significant shifts were detected for the α and β methylene groups in the glutamic residues of the starting material(from 56.2 and 27.76 ppm to 56.47 and 28.04 ppm. New resonances also appeared at the expected positions as a consequence of the enzymatic reaction, thus confirming the structure of the product.

The FTIR spectral analysis showed that, after modification with azo-NH_2, FB-NH_2 and PEG-NH_2, the m-ELRGlu15 presents an amide I band with a maximum close to 1625 cm^{-1}. The displacement of this peak (amide I) to lower frequency (azo, PEG and FB) indicates that the carboxylic groups in the ELR have reacted with the substrate. New peaks also appeared at the expected positions (1164, 976 or 838 cm^{-1} for azo-Glu15; 1342 and 975 cm^{-1} for FB-Glu15; and 1164 and 976 cm^{-1} for PEG-Glu15) as a consequence of the enzymatic reaction, thus confirming the structure of the product.

ELRGlu15

^{1}H NMR (400 MHz, DMSO-d6): δ 8.17 (m, 152H,-NH-Gly(1,3)), 7.91 (m, 68H, -NH-Val(4)), 7.77 (m, 15H, -NH- Glu), 7.58 (m, 68H, -NH- Val(2)), 4.38-4.16 (m, 158H, CH-Val (4)+ α–CH- Pro + αCH-Glu), 4.16-4.04 (m, 68H, CH-Val(2)), 3.73 (m, 304H, -CH_2-Gly

(1,3)), 3.55(m, 150H, δCH_2-Pro), 2.25-2.15 (m, 30H γCH_2-Glu), 2.08-1.66 (m, 466H, γCH2-Pro, C*H*(CH_3)$_2$-Val(2,4), -βCH_2-proline and βCH_2-Glu), 0.91-0.72 (m, 816H, CH(C*H*$_3$)$_2$).

^{13}C NMR (101 MHz, DMSO-d6): δ 174.60, 172.32, 171,84, 171.69, 171.48, 170,41, 170.36, 169,26, 169.12, 59.96, 58.34, 56.10, 52.40, 47.69, 42.49, 41.98, 30.93, 30.61, 29.71, 27.76, 24.89, 19.60, 19.42, 19,37, 18.98, 18.90, 18.83, 18.66, 18.33, 18.13.

FT-IR (ν/cm^{-1}): 3293, 3069, 2966, 2877, 1625, 1520, 1445, 1392, 1372, 1334, 1231, 1028, 929, 873, 667.

MALDI-Tof: 31943 Da

Azo-Glu15

^{1}H NMR (400 MHz, DMSO-d6): δ 8.48-7.43 (m, 339,-NH and CH-arom), 6.86-6.74 (4H, CON*H*-azo), 4.43-4.06 (m, 238H, CH-Val(2,4),)+ α-CH- Pro + αCH-Glu +αCH-Glu-azo), 3.88-3.51 (m, 430H, -CH_2-Gly(1,3) and δCH_2-Pro), 2.33-2.17 (m, 15H γCH_2-Glu), 2.17-1.62 (m, 498H, γCH_2-Pro, C*H*(CH_3)$_2$-Val(2,4), -βCH_2-proline, βCH_2-Glu and γ Glu-azo and βCH_2-Glu-azo), 0.95-0.71 (m, 822H, CH(C*H*$_3$)$_2$).

^{13}C NMR (101 MHz, DMSO-d6): δ 177.91, 173.21, 172.70, 172.33, 171.48, 170.36, 169.12, 169.00, 152.45, 151.53, 147.81, 129.85, 129.44, 127.40, 124.10, 122.74, 119.63, 59.97, 58.35, 56.47, 56.10, (52,4), 47.69, 42.51, 41.99, 30.93, 30.61, 29.70, 25.80, 24.89, 24.70, 19.59, 19.43, 19.27, 18.99, 18.89, 18.81, 18.78, 18.76, 18.73, 18.66, 18.32, 18.14.

FT-IR (ν/cm^{-1}): 3289, 3070, 2966, 2878, 1625, 1524, 1446, 1391, 1372, 1334, 1234, 1164, 1029, 976, 929, 838, 665, 547, 511.

FB-Glu15

^{1}H NMR (400 MHz, DMSO-d6): δ 8.55-7,51 (m, 321H,-NH and CH-arom(FB)), 4.45-4.05 (m, 242H, CH-Val(2,4), α-CH- Pro + αCH-Glu/Glu-FB), 4.01-3.52 (m, 483H, -CH_2-Gly(1,3) +δCH_2-Pro+-CONH-C*H*$_2$_C*H*$_2$(FB)), 2.40-2.23 (m, 19H B(OH)$_2$) 2.23-2.11(m,19H γCH_2-Glu), 2.12-1.62 (m, 556H, γCH_2-Pro, C*H*(CH_3)$_2$-Val(2,4), -βCH_2-proline and βCH_2-Glu/β, γ CH_2Glu-FB), 0.97-0.71 (m, 822H, CH(C*H*$_3$)$_2$).

^{13}C NMR (101 MHz, DMSO-d6): δ 172.32, 172.23, 172.04, 171.84, 171.77, 171.58, 171.48, 170.50, 170.35, 170.22, 169.65, 169.47, 169.12, 168.99, 168.91, 168.83, 168.59, 153.70, 109.99, 107.93, 59.96, 58.34, 56.47, 56.24, 56.10, 55.93, 53.22, 52,67, 49.99, 47.68, 42.50, 42.00, 32.05, 30.92, 30.61, 30.48, 29.70, 28.05, 25.86, 25.55, 24.89, 19.69, 19.59, 19.43, 19.35, 19.02, 18.99, 18.88, 18.83, 18.74, 18.69, 18.65, 18.31, 18.12.

FT-IR (ν/cm^{-1}): 3291, 3070, 2965, 2936, 1626, 1523, 1446, 1392, 1335, 1233, 1028, 975, 929, 835, 667, 540, 523, 507.

PEG-Glu15

^{1}H NMR (400 MHz, DMSO-d6): δ 8.43-7,57 (m, 306H,-NH), 4.37-4.07 (m, 235H, CH-Val (2,4)+ α-CH- Pro + αCH-Glu+-CONH-C*H*$_2$-PEG), 3.89-3.53 (m, 421H, -CH_2-Gly (1,3)+ δCH_2-Pro), 3.51-3.45 (m, 62H, CH_2CH_2O), 3,23-3.20 (s, 5H, OCH_3), 2.14-1.64 (m, 485H, β,γCH_2-Glu, γCH_2-Pro, C*H*(CH_3)$_2$-Val(2,4), -βCH_2-proline and βγCH_2-Glu-PEG), 0.98-0.67 (m, 822H, CH(C*H*$_3$)$_2$).

^{13}C NMR (101 MHz, DMSO-d6): δ 172.32, 171.91, 171.47, 170.36, 170.28, 169.31, 169.11, 168.99, 168.91, 166.76, 70.22, 59.97, 58.34, 56.47, 56.11, 49.86, 47.69, 42.51, 41.99, 30.93, 30.61, 29.70, 27.51, 27.45, 24.89, 19.59, 19.47, 19.43, 19.33, 19.28, 18.99, 18.94, 18.89, 18.83, 18.65, 18.32, 18.12.

FT-IR (ν/cm^{-1}): 3291, 3072, 2966, 2878, 1625, 1524, 1446, 1392, 1372, 1334, 1233, 1164, 1029, 976, 930, 837, 666, 545.

The purity and molecular weight of the polymers were determined from the corresponding MALDI-TOF and SDS-PAGE studies. The results confirmed structural changes in the original ELR as well as the coupling reaction ratio for the different polymers.

SDS-PAGE studies for ELR and m-ELRGlu15 allowed the identification of a major band around 32 kDa, which agrees with the expected molecular weight of the enzymatically modified biopolymer. The intense band observed in SDS-PAGE at around the same value indicated by MALDI–TOF also demonstrates that the purification of m-ELRGlu15 was effective.

To further assess the purity and molecular weight of m-ELRGlu15, matrix-assisted laser desorption/ionization time-of-flight (MALDI-Tof) mass spectrometry was performed using a Voyager STR (Applied Biosystems) in linear mode and with external calibration using bovine serum albumin (BSA).

This technique confirmed the expected modified structure, as shown in Fig. 6, and allowed the coupling ratio to be determined from the weight average (Mw) (34,000-32,000) molecular masses of the products obtained by enzymatic modification.

We collect on Fig. 5 and 6, experimental results of m-ELRGlu15 with highest coupling ratio for the corresponding nucleophile among that obtained through the different assays carried out (data not shown). Best coupling ratio for PEG and FB was obtained with the same condition that has been optimized for phenylazobenzene. Coupling ratio for PEG-Glu15, Azo-Glu15 and FB-Glu15 are 12,8%, 59,5% and 60,8% respectively

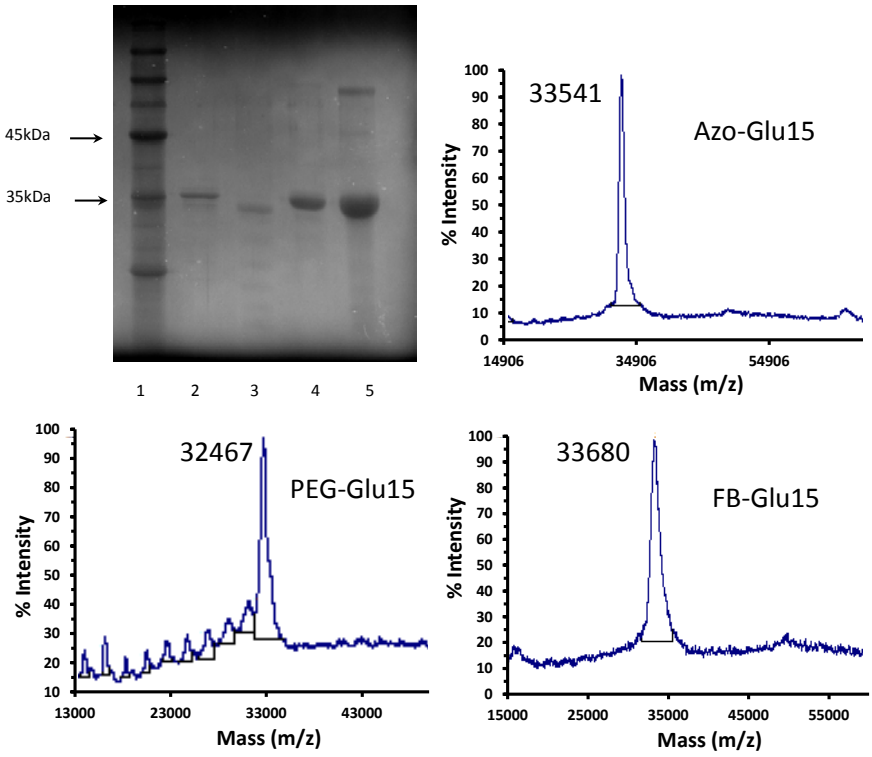

Figure 5. SDS-PAGE and Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF) for mELR. Line 1: Marker; line 2: PEG-Glu15; line 3: Azo-Glu15; line 4: ELR-Glu15; line 5: FB-Glu15

The coupling ratios for m-ELRGlu15s calculated from the MALDI-Tof spectra were very similar to the values calculated by ^{1}H NMR analysis and/or from absorbance measurements.

The thermal transitions for ELRGlu15 and m-ELRGlu15s were determined by **differential scanning calorimetry** (DSC) **analysis** using a Mettler Toledo 822^{e} DSC instrument, as described in the Materials and Methods section. The DSC curves for aqueous solutions of these polymers at 50 mg mL^{-1} prepared at pH 2 are shown in Fig. 6. It can be seen from this figure that the DSC endotherms for all m-ELRGlu15s prepared are similar, thus confirming that the modified biopolymers conserve their smart nature.

The conjugated Azo-Glu15 and FB-Glu15 groups decrease the transition temperature of the original polymer from 27.4 °C to 22.3 °C and 10.7 °C, respectively, due to the effect of these aromatic groups on the global hydrophobicity of the polymer [5]. More hydrophobic polymers have higher ΔH values due to the increase in the number of water molecules dedicated to hydrophobic hydration, which lowers the transition temperature.

DSC analysis of PEG-Glu15 showed that the transition temperature is higher than for the original biopolymer due to a decrease in global hydrophobicity caused by the PEG unit.

Figure 6. DSC endotherms (5 °C/min after 10 min at 5 °C) for aqueous solutions of the elastin-like recombinamer (ELRGlu15) and the enzymatically modified elastin-like recombinamer (m-ELR) at pH 2 and 50 mg•mL^{-1} during the first heating scan.