Research Letter

Charge Transport Phenomena in Peptide Molecular Junctions

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Inelastic electron tunneling spectroscopy (IETS) is a valuable in situ spectroscopic analysis technique that provides a direct portrait of the electron transport properties of a molecular species. In the past, IETS has been applied to small molecules. Using self-assembled nanoelectronic junctions, IETS was performed for the first time on a large polypeptide protein peptide in the phosphorylated and native form, yielding interpretable spectra. A reproducible 10-fold shift of the I/V characteristics of the peptide was observed upon phosphorylation. Phosphorylation can be utilized as a site-specific modification to alter peptide structure and thereby influence electron transport in peptide molecular junctions. It is envisioned that kinases and phosphatases may be used to create tunable systems for molecular electronics applications, such as biosensors and memory devices.

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1. INTRODUCTION

The incorporation of biomolecules into nanoscale molecular junctions has become an area of intense research relevant to molecular electronics [1, 2]. Nevertheless, understanding electron transport through the complex structure of biomolecules has been difficult, and at times contradictory [3, 4]. Inelastic electron tunneling spectroscopy (IETS) is a powerful nanoscopic tool for characterizing the interaction between tunneling electrons and discrete molecular vibrations along the pathway of conduction [5]. Recently, IETS has been used to identify incorporated molecular species [6], chemical interactions at the metal-molecule interface [7], orientation of the molecule [8], and the orbital pathway followed by tunneling electrons [9]. While electron transport properties of proteins have been a matter of interest for some time [10–13], IETS has never been applied to the characterization of molecular junctions containing large protein polypeptides. Here, we investigate whether IETS is applicable to polypeptide-based molecular junctions and can detect physiologically relevant posttranslational modifications of a peptide such as phosphorylation. In this study, we incorporate a peptide and its phosphorylated derivative in a well-described molecular junction architecture [7, 14] and characterize them using IETS and I/V analysis.

2. RESULTS AND DISCUSSION

A peptide was derived from Ca2+/calmodulin (CaM)-dependent protein kinase II (CaM kinase II), an important mediator of Ca2+ signaling pathways in cells. Phosphorylation of Thr 286 induces a conformational shift that frees this protein from Ca2+-mediated activation [6]. An eleven amino acid sequence from the regulatory domain of CaM kinase II (amino acids 281–291) was utilized as a test peptide. As a basis for in silico structural studies, the coordinates for amino acids 281–291 were obtained from the crystallographic structure of CaM kinase II PDB 2bdw [15].

Thr 286 was found within the helical portion of the isolated test [15]. After substituting Ile 281 with Met and
Asp 285 with Glu, energy minimization was performed using conjugate gradient method on the following amino acid sequence: Met-His-Arg-Gln-Glu-Thr-Val-Asp-Cys-Leu-Lys. In the minimized structure, the side chain of Thr 286 was phosphorylated and the modified peptide was further minimized using the same algorithm (CHARMM force field [16]). The minimization was carried out with no solvent and with the implicit solvent represented by distance-dependent dielectric. The nonphosphorylated test peptide is a combination of random coil and alpha helical secondary structural elements (Figure 1(a)). The model for the phosphorylated peptide (Figure 1(b)) revealed a marked conformational alteration. The interatom distance between C(b) of Arg 283 and O(g) of Thr 286 in the nonphosphorylated peptide is 3.310 Å, while it is 5.214 Å in the phosphorylated peptide, an increased distance of 1.904 Å.

The test peptide was characterized using a self-assembled metal-peptide-metal junction [14]. The peptide’s cysteine provided a thiol moiety for linkage to the gold electrode [17, 18]. The difference in molecular weight between nonphosphorylated and phosphorylated peptides is 5.5%, and the difference in molecular volume for the minimized conformations of the two forms of the peptide is in the range of 3 to 8%, depending on the way the volume is calculated. Despite the conformational dissimilarity, the incremental changes in volume and overall shape of the peptide lead to very similar packing densities on the electrode. The estimated area of contact in a microsphere junction is...
The “footprint” of a single CaM Kinase II peptide molecule on an electrode is 1–3 nm², depending on the orientation, thus 20 to 60 molecules (either nonphosphorylated or phosphorylated) can be attached to a 60 nm² surface area.

Magnetic arrays were cleaned as previously described [19]. Arrays were modified with CaM kinase (MHRQETVDClK, Anaspec, San Jose, CA) and phosphorylated CaM kinase (MHRQEpTVDClK, Anaspec, San Jose, CA) by incubating in 2 mL of 10 μM solution in MilliQ water at 4°C for a duration not less than 24 hours. The substrates were then rigorously rinsed in MilliQ water followed by drying. Using magnetic assembly, metallized spheres were deposited at the source/drain gap, completing the electrical circuit through the peptides. Incorrectly seated assemblies could be detected by metal-metal contacts, which were easily excluded from the data set (not shown).

The assembly was transferred to a cryogenic vacuum probe station using a parametric analyzer (Agilent 4155B, Palo Alto, CA) under computer control for I/V and IETS as previously described [19]. IETS provided a means to measure the vibrational modes of the metal-protein-metal junction [19–22]. These studies were performed at 4 K with standard ac modulation techniques combined with two lock-in amplifiers to record the second harmonic signal (d²I/dV²) which was then normalized by the differential conductance (dI/dV) to yield the IET spectrum of junctions containing either the nonphosphorylated or the phosphorylated peptides (Figure 2). Fourier transform infrared spectra (FT-IR) were obtained on a spectrum RX I FT-IR spectrometer (PerkinElmer, Wellesley, MA) at a resolution of 4 cm⁻¹ at room temperature. Reference spectra of air were recorded and subtracted from the sample spectra. CaM kinase II derived peptides were dissolved in anhydrous isopropanol (100 μg/mL). The solution was dried on the surface of KBr plates under N₂.

The amide I, II, and III bands, components of a peptide’s backbone structure, are present within both the IETS and FT-IR spectra, as shown in IETS peak 3 [23]. IETS peak 6 encompasses a number of modes detected via FT-IR, including the amide A and amide B bands [23]. Amino acid side chains also contribute to the IET spectra.
Within IETS peak 3 are expected modes for a number of amino acid side chains, including Asp, Glu, Gln, Arg, Lys, and His, the peaks of which overlap with each other as well as elements of the peptide backbone. IETS peak 4 accounts for the S–H stretching mode of cysteine, which absorbs in a spectral region free from overlapping by other groups [23].

The marked vibrational intensity of the IETS S–H vibrational mode, compared to the lack of a prominent FT-IR peak, is attributable to this functional group being the point of linkage for the peptide on the metal (Au) electrode. All electrons injected into the peptide must exit via the gold–sulfur linkage on the electrode surface making this single bond prominent in IETS. For the phosphopeptide, a peak at 1039 cm$^{-1}$ consistent with the P–OC stretch within a phosphate group is detected by FT-IR [24]. In the IET spectra, there is a slight red shift in frequency in peak 2 in the nonphosphorylated peptide when compared to the phosphorylated peptide that matches the location of the P–OC stretch detected by FT-IR. Taken together, the IETS results for the phosphorylated and nonphosphorylated peptides indicate that the peptide backbone and side chains contribute to electron tunneling in the peptide.

I–V traces of the nonphosphorylated CaM kinase II-derived peptide were acquired on 74 devices, yielding an average of 62 nA at 0.5 V bias (Figure 3(a)). The distribution of current values at 500 mV showed a major peak (Figure 3(b)). Proposed mechanisms of transport through alpha helices include: (1) the electrostatic fields created by the dipole moment of peptide helices; (2) $\pi$ orbitals of the peptide backbone present within helices; and (3) through hydrogen bonds that contribute to the structural stability of helices [11, 13, 25–27]. Electron transport through phosphorylated CaM kinase II-derived peptide was reproducibly observed in a large number (62) of molecular electronics devices (Figure 3(c)). The reproducible average current value at 0.5 V was 6 nA, an order of magnitude less than that found with the nonphosphorylated peptide (62 nA).

The distribution of current values at 500 mV showed a major peak (Figure 3(d)) and electron transport through the immobilized phosphorylated peptide was very reproducible (Figure 3(e)). Helix denaturization has previously been shown to alter the rate of electron transport in a dichromophoric peptide model, resulting in an order of magnitude difference in electron transport [28]. A reproducible 10-fold difference in electron transport between the phosphorylated and nonphosphorylated form of CaM kinase II-derived peptide is shown in Figure 3(f). Since the calculated packing densities of the two forms of the peptide are very similar and thus the number of molecules analyzed in each junction is comparable, this difference is likely attributable to a conformational shift within the secondary structure of the peptide.

3. CONCLUSION

This work can be highly relevant to the field of molecular electronics. These data indicate that seemingly minor post-translational modifications of a protein polypeptide can have profound effects on the electron transport properties revealed by IET spectra and I/V characteristics. Long term, the field of protein electronics may yield new classes of biomaterial sensors, computing devices, and high-throughput screening tools for kinase-targeted pharmaceuticals.

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REFERENCES


