Hindawi Publishing Corporation
Research Letters in Nanotechnology
Volume 2008, Article ID 853253, 5 pages
doi:10.1155/2008/853253

Research Letter

Charge Transport Phenomena in Peptide Molecular Junctions

Alessandra Luchini,[1,2] David P. Long,[3] Iosif I. Vaisman,[4] Emanuel F. Petricoin,[2] David H. Geho,[2] and Lance A. Liotta[2]

[1] *CRO-IRCCS National Cancer Institute, 33081 Aviano, Italy*
[2] *Center for Applied Proteomics and Molecular Medicine, College of Arts and Sciences, George Mason University, Manassas, VA 20110, USA*
[3] *Research and Development Center, Science Applications International Corporation (SAIC), 9460 Innovation Drive, Manassas, VA 20110, USA*
[4] *Department of Bioinformatics and Computational Biology, School of Computational Sciences, George Mason University, Manassas, VA 20110, USA*

Correspondence should be addressed to Alessandra Luchini, aluchini@gmu.edu

Received 23 December 2007; Accepted 10 February 2008

Recommended by Federico Rosei

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.

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 Ca^{2+}/calmodulin (CaM)-dependent protein kinase II (CaM kinase II), an important mediator of Ca^{2+} signaling pathways in cells. Phosphorylation of Thr 286 induces a conformational shift that frees this protein from Ca^{2+}-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

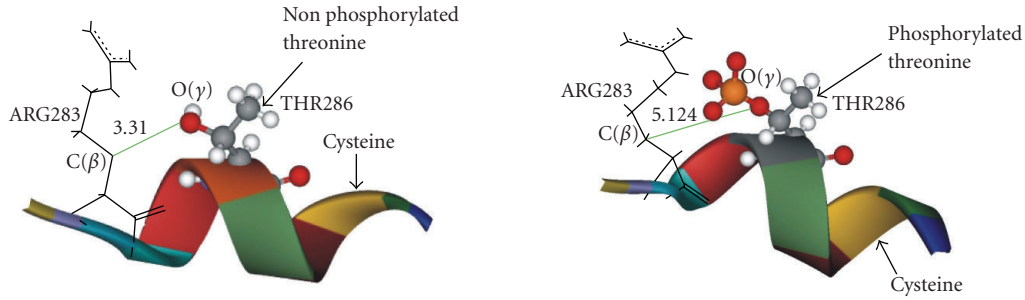

Figure 1: (a) Nonphosphorylated CaM kinase II-derived peptide and (b) phosphorylated peptide. Ribbon, ball and stick, and wireframe 3D structure representations.

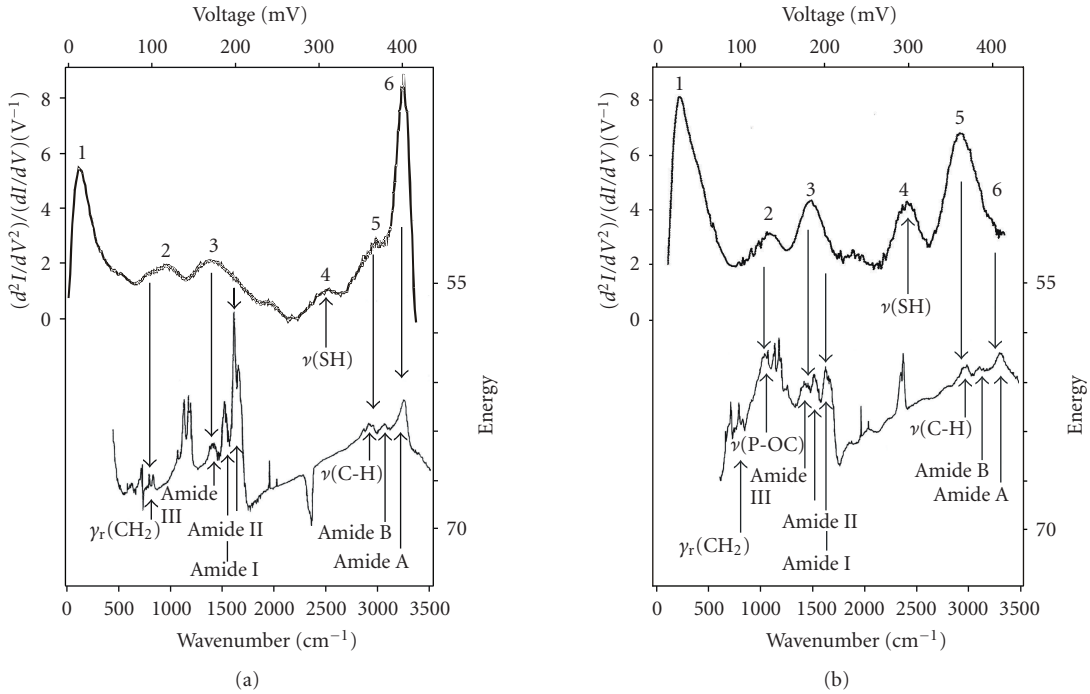

Figure 2: Characteristic IETS spectra (bolder line) for the nonphosphorylated (a) and phosphorylated (b) peptides. FTIR results of each peptide are shown beneath the IET spectra.

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

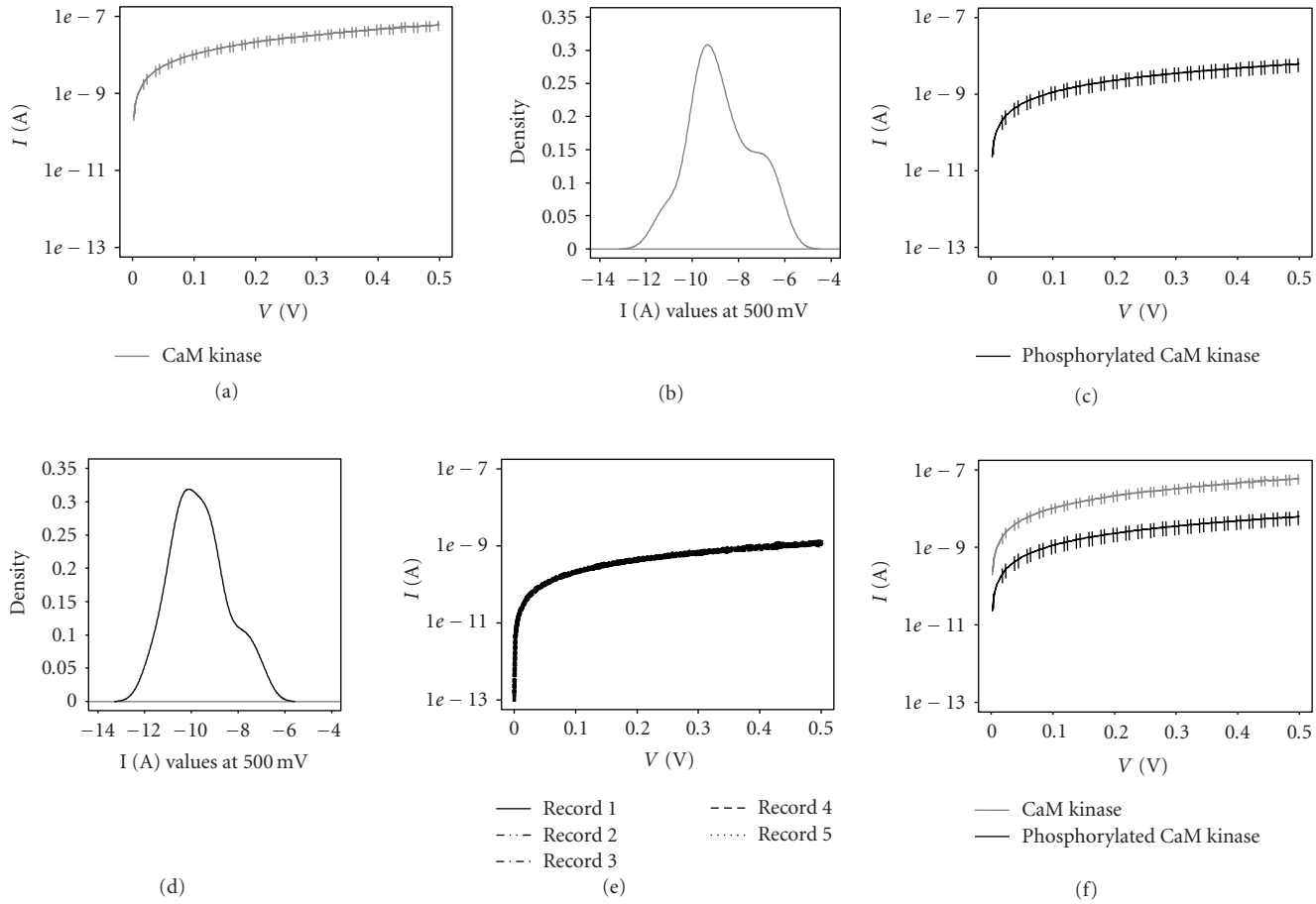

Figure 3: (a) I/V properties of the molecular junctions containing the nonphosphorylated test peptide (log scale, mean and standard errors); (b) density plot of I values (A) at 0.5 V for the nonphosphorylated peptide, logarithmic scale; (c) I-V characteristics of the molecular junctions containing the phosphorylated test peptide, logarithmic scale, mean and standard errors; (d) density plot of I values (A) at 0.5 V, logarithmic scale; (e) five separate I-V traces of a molecular junction containing the phosphopeptide; (f) a comparison of the average I-V traces for plain (gray) and phosphorylated (black) test peptides (Wilcoxon rank sum $p < 0.0001017$).

60 nm^2. The "footprint" of a single CaM Kinase II peptide molecule on an electrode is 1–3 nm^2, depending on the orientation, thus 20 to 60 molecules (either nonphosphorylated or phosphorylated) can be attached to a 60 nm^2 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 (d^2I/dV^2) 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 N_2.

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) π 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 dichromomorphic 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 biomedical sensors, computing devices, and high-throughput screening tools for kinase-targeted pharmaceuticals.

ACKNOWLEDGMENTS

The authors appreciate the generous support of Dr. Vikas Chandhoke, Mr. Tom Huff, Dr. Ranganathan Shashidhar, Dr. Enrico Garaci, Dr. Alfonso Colombatti, Dr. Claudio Belluco, Dr. Barney Bishop, Ms. Virginia Espina, and Dr. Victor Morozov. This work was partly supported by the Italian Istituto Superiore di Sanità in the framework of Italy/USA HHS cooperation agreement, George Mason University, and the Italian Ministry of Public Health.

REFERENCES

[1] J. M. Tour, *Molecular Electronics: Commercial Insights, Chemistry, Devices, Architectures and Programming*, World Scientific, River Edge, NJ, USA, 2003.

[2] R. L. Carroll and C. B. Gorman, "The genesis of molecular electronics," *Angewandte Chemie International Edition*, vol. 41, no. 23, pp. 4378–4400, 2002.

[3] H. Cohen, C. Nogues, R. Naaman, and D. Porath, "Direct measurement of electrical transport through single DNA molecules of complex sequence," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 33, pp. 11589–11593, 2005.

[4] M. Taniguchi and T. Kawai, "DNA electronics," *Physica E*, vol. 33, no. 1, pp. 1–12, 2006.

[5] A. Troisi and M. A. Ratner, "Molecular signatures in the transport properties of molecular wire junctions: what makes a junction "molecular"?" *Small*, vol. 2, no. 2, pp. 172–181, 2006.

[6] A. Hudmon and H. Schulman, "Neuronal Ca^{2+}/calmodulin-dependent protein kinase II: the role of structure and autoregulation in cellular function," *Annual Review of Biochemistry*, vol. 71, pp. 473–510, 2002.

[7] D. P. Long, J. L. Lazorcik, B. A. Mantooth, et al., "Effects of hydration on molecular junction transport," *Nature Materials*, vol. 5, no. 11, pp. 901–908, 2006.

[8] A. Troisi, J. M. Beebe, L. B. Picraux, et al., "Tracing electronic pathways in molecules by using inelastic tunneling spectroscopy," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 36, pp. 14255–14259, 2007.

[9] R. C. Jaklevic and J. Lambe, "Molecular vibration spectra by electron tunneling," *Physical Review Letters*, vol. 17, no. 22, pp. 1139–1140, 1966.

[10] A. Szent-Györgyi, "Towards a new biochemistry?" *Science*, vol. 93, no. 2426, pp. 609–611, 1941.

[11] E. Galoppini and M. A. Fox, "Effect of the electric field generated by the helix dipole on photoinduced intramolecular electron transfer in dichromophoric α-helical peptides," *Journal of the American Chemical Society*, vol. 118, no. 9, pp. 2299–2300, 1996.

[12] I. Hwang, T. Thorgeirsson, J. Lee, S. Kustu, and Y.-K. Shin, "Physical evidence for a phosphorylation-dependent conformational change in the enhancer-binding protein NtrC," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 9, pp. 4880–4885, 1999.

[13] S. S. Isied, M. Y. Ogawa, and J. F. Wishart, "Peptide-mediated intramolecular electron transfer: long-range distance dependence," *Chemical Reviews*, vol. 92, no. 3, pp. 381–394, 1992.

[14] D. P. Long, C. H. Patterson, M. H. Moore, D. S. Seferos, G. C. Bazan, and J. G. Kushmerick, "Magnetic directed assembly of molecular junctions," *Applied Physics Letters*, vol. 86, no. 15, Article ID 153105, 3 pages, 2005.

[15] O. S. Rosenberg, S. Deindl, R.-J. Sung, A. C. Nairn, and J. Kuriyan, "Structure of the autoinhibited kinase domain of CaMKII and SAXS analysis of the holoenzyme," *Cell*, vol. 123, no. 5, pp. 849–860, 2005.

[16] A. D. MacKerell Jr., D. Bashford, M. Bellott, et al., "All-atom empirical potential for molecular modeling and dynamics studies of proteins," *Journal of Physical Chemistry B*, vol. 102, no. 18, pp. 3586–3616, 1998.

[17] T. Morita, S. Kimura, S. Kobayashi, and Y. Imanishi, "Photocurrent generation under a large dipole moment formed by self-assembled monolayers of helical peptides having an *N*-ethylcarbazolyl group," *Journal of the American Chemical Society*, vol. 122, no. 12, pp. 2850–2859, 2000.

[18] A. Ulman, *An Introduction to Ultrathin Organic Films*, Academic Press, San Diego, Calif, USA, 1991.

[19] J. G. Kushmerick, J. Lazorcik, C. H. Patterson, R. Shashidnar, D. S. Seferos, and G. C. Bazan, "Vibronic contributions to charge transport across molecular junctions," *Nano Letters*, vol. 4, no. 4, pp. 639–642, 2004.

[20] W. H. Weinberg, "Inelastic electron tunneling spectroscopy: a probe of the vibrational structure of surface species," *Annual Review of Physical Chemistry*, vol. 29, pp. 115–139, 1978.

[21] L. Cai, M. A. Cabassi, H. Yoon, et al., "Reversible bistable switching in nanoscale thiol-substituted oligoaniline molecular junctions," *Nano Letters*, vol. 5, no. 12, pp. 2365–2372, 2005.

[22] W. Wang, T. Lee, I. Kretzschmar, and M. A. Reed, "Inelastic electron tunneling spectroscopy of an alkanedithiol self-assembled monolayer," *Nano Letters*, vol. 4, no. 4, pp. 643–646, 2004.

[23] A. Barth and C. Zscherp, "What vibrations tell us about proteins," *Quarterly Reviews of Biophysics*, vol. 35, no. 4, pp. 369–430, 2002.

[24] M. Silverstein, F. X. Webster, and D. J. Kiemle, *Spectrometric Identification of Organic Compounds*, John Wiley & Sons, Hoboken, NJ, USA, 2005.

[25] H. B. Gray and J. R. Winkler, "Electron tunneling in structurally engineered proteins," *Journal of Electroanalytical Chemistry*, vol. 438, no. 1-2, pp. 43–47, 1997.

[26] W. G. Hol, "The role of the α-helix dipole in protein function and structure," *Progress in Biophysics and Molecular Biology*, vol. 45, no. 3, pp. 149–195, 1985.

[27] J. Halpern and L. E. Orgel, "The theory of electron transfer between metal ions in bridged systems," *Discussions of the Faraday Society*, vol. 29, pp. 32–41, 1960.

[28] M. A. Fox and E. Galoppini, "Electric field effects on electron transfer rates in dichromophoric peptides: the effect of helix unfolding," *Journal of the American Chemical Society*, vol. 119, no. 23, pp. 5277–5285, 1997.