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Positively charged peptides can interact with each other, as revealed by solid phase binding assays

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Abstract

Solid phase assay systems such as enzyme-linked immunosorbent assay (ELISA), surface plasmon resonance (SPR), and overlay gels are used to study processes of protein–protein interactions. The common principle of all these methods is that they monitor the binding between soluble and surface-immobilized molecules. Following the use of bovine serum albumin (BSA)–peptide conjugates or isolated synthetic peptides and the above-mentioned solid phase assay systems, the results of the current work demonstrate that positively charged peptides can interact with each other. Both the ELISA and SPR methods demonstrated that the binding process reached saturation with K_d values ranging between 1 and 14 nM. No interaction was observed between BSA conjugates bearing positively charged peptides and conjugates bearing negatively charged peptides or with pure BSA molecules, strengthening the view that interaction occurs only between positively charged peptides. However, interactions between peptides in solution were not observed by nuclear magnetic resonance (NMR) or by native gel electrophoresis. It appears that for positively charged molecules to interact, one of the binding partners must be immobilized to a surface, a process that may lead to the exposure of otherwise masked groups or atoms. We discuss the relevance of our findings for the use of solid phase assay systems to study interactions between biomolecules.

Keywords: Positively charged peptides; ELISA; SPR; NMR; Protein–protein interactions

Protein–protein interactions are involved in nearly every aspect of cell life, including processes such as differentiation, progression of the cell cycle, and the response of cells to their environment and various pathogens [1,2]. Especially now, in the age of genomics and proteomics, there is a growing interest in methods that will enable the study of how proteins interact among themselves or with other macromolecules, such as carbohydrates and nucleic acids.

Since it was first developed and described, the enzyme-linked immunosorbent assay (ELISA)[1] technique has been widely and extensively applied as an immunoassay to study

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[1] *Abbreviations used*: ELISA, enzyme-linked immunosorbent assay; SPR, surface plasmon resonance; NPC, nuclear pore complex; NLS, nuclear localization signal; BSA, bovine serum albumin; PBS, phosphate-buffered saline; HPLC, high-performance liquid chromatography; Bb, biotinylated BSA; HRP, horseradish peroxidase; OD, optical density; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; TOCSY, total correlation spectroscopy; ROESY, rotating frame Overhauser effect spectroscopy; TPPI, time proportional phase incrementation; RU, resonance units; EDTA, ethylenediaminetetraacetic acid; NES, nuclear export signal; NMR, nuclear magnetic resonance; ATR–FTIR, attenuated total reflection–Fourier transform infrared.

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the interaction between antibodies and antigens, especially for diagnostic purposes in the medical field. However, due to its great sensitivity, its ease of use, and the reproducibility of its results, ELISA has also been used to study various aspects of protein–protein and protein–carbohydrate interactions [3,4].

ELISA is a solid phase assay in which one of the interacting partners is immobilized on a surface, in most cases polystyrene [5], while its counteracting partner is added as a soluble entity. This principle of binding soluble and immobilized protein molecules is the basis of several other methods that are currently being used for the analysis of in vitro binary protein interactions. Surface plasmon resonance (SPR) has also been applied to study the interaction among a variety of molecules such as receptors, antibodies, antigens, enzymes, nucleic acids, drugs, cells, and viruses. SPR systems enable continuous monitoring of the formation of complexes with added soluble molecules and dissociation from the immobilized ligand [6]. The blot overlay technique has also long been used to directly visualize protein–protein interactions and the formation of protein complexes. In fact, results obtained from overlay gel assays, alone or in conjugation with other techniques such as size exclusion chromatography and crosslinking, have suggested that under native conditions many macromolecules exist as supramolecular assemblies [7]. In our laboratory, we have used ELISA, SPR, and blot overlay gels to study various aspects related to the nuclear import of HIV-1 karyophilic proteins [8–11].

Translocation of molecules across the nuclear envelope occurs via the nuclear pore complex (NPC) and is governed by cellular proteins known as importins and exportins that mediate import and export, respectively [12]. The interaction between actively imported proteins and their cellular receptors (importins) is mediated by a nuclear localization signal (NLS). NLSs are short amino acid stretches that usually contain positively charged residues such as lysine and arginine [13]. Bovine serum albumin (BSA) molecules that bear covalently attached synthetic NLS peptides are routinely used as nuclear import transport substrates when permeabilized cultured cells are used as an in vitro nuclear import assay system [8,9]. ELISA-based systems have been instrumental in studying protein domains and peptides that mediate the binding of karyophilic proteins or BSA–NLS conjugates to their cellular nuclear import receptors [14]. BSA–SV40–NLS and BSA–Rev–NLS binding to importin α and importin β, respectively, have been demonstrated previously by us [9,10] and others [15–17]. The ELISA system has also been used by us to screen for peptides that bind specifically to the NLS domain of HIV-1 karyophilic proteins, that is, anti-NLS peptides that eventually will block NLS-mediated nuclear import [11,18]. During the course of these experiments, we observed that positively charged BSA–NLS conjugates, which are expected to repulse each other, unexpectedly interact among themselves. This unpredicted binding could be competitively inhibited and was found to be mediated by the NLS peptide, indicating a specific interaction.

Materials and methods

Buffers

The following buffers were used: maleic acid (10 mM maleic acid, 150 mM NaCl, pH 3.0), acetate (10 mM acetic acid, 150 mM NaCl, pH 4.5), phosphate-buffered saline (PBS, 50 mM Na_2HPO_4, 100 mM NaCl, pH 7.4), tricine (10 mM tricine, 150 mM NaCl, pH 8.0), carbonate (0.05 M Na_2CO_3/0.05 M $NaHCO_3$, pH 9.6) and phosphate (10 mM Na_2HPO_4, 100 mM NaCl, pH 12.0).

Synthetic peptides

Peptides were purchased from GL-Biochem (China). The peptide sequences are given in Table 1. All peptides

Table 1
Summary of the results obtained with the BSA–peptide conjugates used in the current study

Peptide name	Amino acid sequence	Charge	Derived from:	Apparent K_d for peptide–peptide binding[a] (nM)
SV40	PKKKRKV	+5	SV40 T-antigen	1
SV40 (reverse)	VKRKKKP	+5		1
SV40 (mut)	PKKTRKV	+4		1
SV40 (reverse mut)	VKRTKKP	+4		1
Tat	GRKKRRQRRRAHQN	+9	HIV-1 transcription activation protein	1
Rev	RQARRNRRRRWR	+8	HIV-1 nucleocytoplasmatic shuttling protein	1
Rev (reverse)	RWRRRRNRRAQR	+8		1
Vip	KRILANRQSAARSKERKIR	+7	Nuclear import of agrobacterium T-DNA	2.5
Vip (reverse)	RIKREKSRAASQRNALIRK	+7		2.5
CP	KRPGDIIISTPVSKVRRR	+5	TYLCV structural viral protein	10
BV1	KIEPNRSRSYIKLKRLRFKGTVK	+8	Gemini virus movement protein	10
H2A	SGRGKQGGKARAKAKTRSSR	+8	Histone	5
p53	MFRELNEALELKDAQA	−2	Tumor suppressor	No signal detected
VprN	NEWTLELLEELKNEAVRHF	−2	HIV-1 responsible for cell cycle arrest	No signal detected

[a] The apparent K_d values were measured, using the ELISA system, between a BSA–peptide conjugate and the same corresponding Bb–peptide conjugate containing the designated peptide.