

COMPARISON OF THE INTRINSIC KINASE ACTIVITY AND SUBSTRATE SPECIFICITY OF C-ABL AND BCR-ABL

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Abstract: We studied the intrinsic tyrosine kinase activity and substrate specificity of c-Abl and Bcr-Abl protein tyrosine kinases (PTKs) using the peptide substrates discovered from a synthetic combinatorial peptide library. Our data indicate that the phosphorylation of these peptides by Bcr-Abl was consistently stronger than that by c-Abl. Bcr-Abl also showed substrate preference towards those peptides with one or more positive charges. © 1998 Elsevier Science Ltd. All rights reserved.

Introduction

Tyrosine kinase and transforming activities of the *c-abl* proto-oncogene can be activated by two different mechanism: (1) integration into the viral genome and (2) translocation of *bcr* sequences. The *v-abl* oncogene was discovered in the genome of the Abelson virus.¹ The protein product of *v-abl* has been shown to have much higher tyrosine kinase activity than its normal cellular counterpart, c-Abl. The SH3 domain of c-Abl protein is deleted in v-Abl protein. It is generally believed that the SH3 domain has a negative regulatory effect on the catalytic domain of c-Abl PTK.^{2,3} Deletion of the SH3 domain leads to activation of *in vivo* kinase activity and transforming potential of c-Abl.⁴ The *bcr-abl* is a chimeric oncogene first discovered in Philadelphia chromosome-positive leukemia patients.⁵ Evidence suggests that the oncogenic activity of Bcr-Abl chimeric protein is due to its elevated tyrosine kinase activity as a result of the fusion of the Bcr sequence to the N-terminus of Abl protein.⁶ In contrast to v-Abl, Bcr-Abl retains an intact SH3 domain, suggesting that the unique mechanism conferred by the Bcr sequence is responsible for activation of the tyrosine kinase activity of Bcr-Abl. Recently, we generated a synthetic combinatorial peptide library using the “one-bead one-peptide” concept^{7,8} and screened the library against c-Abl PTK to identify linear peptide substrates. Fourteen peptides grouped into three distinct motifs were identified.⁹ Details of the screening method and results will be reported in a different publication.¹⁰ In this report, we compared the intrinsic tyrosine kinase activity and substrate specificity of c-Abl and Bcr-Abl using those discovered peptides as substrates.

Materials and Methods

Partially purified human c-Abl and Bcr-Abl were kindly provided by Drs. Owen N. Witte and Daniel E. H. Afar at University of California at Los Angeles. The synthetic combinatorial peptide library was synthesized

by a “split synthesis” approach as described previously.⁷ Briefly, polyethylene glycol grafted polystyrene beads or TentaGel (TG) beads were chosen as solid-phase support. Standard solid-phase peptide synthesis method with fluorenylmethyloxycarbonyl (Fmoc) chemistry was used to synthesize the peptide library.¹¹ The peptide library synthesized on TG beads was a dedicated octapeptide library (XXIYXXXX-TG), wherein X represents one of twenty natural amino acid except cysteine and the third and fourth positions from the amino-terminus were kept constant as L-isoleucine (I) and L-tyrosine (Y), respectively. Two million peptide-beads, representing approximately 4% of the library scope, were screened for substrates using c-Abl PTK as described previously.^{12–}

¹⁴ In brief, the peptide-bead library was incubated with [γ -³²P]ATP and c-Abl PTK. After incubation, the peptide-bead library was thoroughly washed and immobilized on the glass plates with agarose. ³²P-labeled beads were localized by autoradiography and individual labeled beads were isolated for microsequencing. The identified peptides were resynthesized and tested for their activity against both c-Abl and Bcr-Abl using the method as described previously.^{13–15}

Results and Discussion

Comparison of in vitro Phosphorylation of Peptides by c-Abl and Bcr-Abl: Fourteen peptides were identified by screening the peptide-bead library with c-Abl PTK. Figure 1 shows phosphorylation of these peptides by partially purified c-Abl and Bcr-Abl PTKs. When compared to angiotensin II, these peptides were much more efficient substrates for both c-Abl and Bcr-Abl PTKs (data not shown). However, the levels of ³²P incorporation into these peptides by Bcr-Abl PTK were consistently 2- to 9-fold higher than that by c-Abl PTK, suggesting that the in vitro kinase activity of Abl tyrosine kinase is activated by the fusion of the Bcr sequence to N-terminus of c-Abl. Since these two PTKs used in the present experiments are purified from Sf9 cells in which either c-Abl or Bcr-Abl are hyperexpressed, it is unlikely that enough cellular regulatory factors are copurified with these two proteins and stimulate the kinase activity of Bcr-Abl PTK other than c-Abl. It appears that the Bcr sequence may activate the tyrosine kinase activity of Abl in a direct manner. Although our data can not distinguish if activation of Abl by Bcr is intramolecular or intermolecular, we hypothesize that the Bcr sequence may interact with the regulatory and/or catalytic domains of Abl and induce a specific conformational change which enhances the tyrosine kinase activity of the Abl catalytic domain. The hypothesis is supported by our observation that Bcr-Abl PTK, when compared to c-Abl PTK, has shown a differentiated substrate preference towards some of these peptides. Table 1 shows the amount of ³²P-incorporation into these fourteen peptides at a concentration of 100 μ M in 30 min. The ratios of phosphorylation by Bcr-Abl PTK to that by c-Abl PTK were compared. These fourteen peptides can be divided into two groups (Table 1) with Bcr-Abl/c-Abl phosphorylation ratios ranging from 2.0 to 4.0 (group I) and from 5.0 to 9.0 (group II). When comparing to phosphorylation of the same peptides by c-Abl PTK, Bcr-Abl seems to prefer the second group of peptides more than the first group of peptides. Although there is no strong consensus among the two groups of peptides, all the

peptides in group II except DDIYEIYW have one to four basic residues (arginine, lysine, or histidine) and five of the seven peptides have one to three arginine residues. In contrast, none of the peptides in group I has any arginine, lysine, or histidine. Instead, all the peptides in group I have two to four acidic residues (glutamic acid or aspartic acid). These data suggest that peptide substrate preference between c-Abl and Bcr-Abl PTKs may be somewhat different although Bcr-Abl/c-Abl phosphorylation ratios in either Group I or Group II were not uniform.

Comparison of K_m and V_{max} of Peptide Substrates between c-Abl and Bcr-Abl: The six best peptide substrates for c-Abl and Bcr-Abl PTKs (Figure 1) were chosen for determination of kinetic parameters. Table 2 shows the comparison of K_m and V_{max} values of the six peptide substrates between c-Abl and Bcr-Abl PTKs. The K_m values of EEIYEPPY, EEIYDYAY, EEIYAEWF, and EEIYYYVH for both c-Abl and Bcr-Abl PTKs were very similar. The K_m value of ERIYARTK for c-Abl PTK was approximately 2-fold higher than that for Bcr-Abl while the K_m value of EEIYDEIY for c-Abl PTK was roughly half of that for Bcr-Abl. In comparison, the V_{max} values of all six peptide substrates for Bcr-Abl PTK were always 7- to 13-fold higher than that for c-Abl. The ratios of V_{max} to K_m of all six peptides for Bcr-Abl PTK were 5- to 17-fold higher than that for c-Abl PTK. The kinetic analysis also suggests that the fusion of the Bcr domain to c-Abl may result in a conformational change of the enzyme active site leading to a higher enzyme activity as well as some changes in the substrate specificity.

Substrate Specificity Determinants in EEIYEPPY for c-Abl and Bcr-Abl: To compare substrate specificity determinants within individual peptides between c-Abl and Bcr-Abl PTKs, alanine scan was carried out for EEIYEPPY which was one of the best peptide substrates for both c-Abl and Bcr-Abl PTKs (Figure 2). It was evident that the overall phosphorylation profiles of different peptides between these two PTKs were very similar although the absolute phosphorylation was different. If phosphorylation of the parent compound (EEIYEPPY) by both c-Abl and Bcr-Abl was considered as controls (100%), the percentage phosphorylation by both c-Abl and Bcr-Abl PTKs was very close during alanine scan. For instance, when the first glutamic acid (Glu-1) from N-terminus was replaced by alanine, ^{32}P -phosphate incorporation into AEIYEPPY by c-Abl decreased from 1.83 pmol (control) to 0.22 pmol (88%); ^{32}P -phosphate incorporation into AEIYEPPY by Bcr-Abl decreased from 7.42 pmol (control) to 0.67 pmol (91%). The replacement of Glu-5 by alanine resulted in a increase of phosphorylation by both c-Abl (66%) and Bcr-Abl (50%). Similar results were obtained when the other amino acids within EEIYEPPY were substituted by alanine. Although the V_{max} value of EEIYEPPY for Bcr-Abl PTK was 8-fold higher than that for c-Abl PTK (Table 2), the SAR profiles of EEIYEPPY for both c-Abl and Bcr-Abl PTKs were very similar. Alanine scan on EEIYEPPY indicates that E_IYA_P_ is the preferred motif for both c-Abl and Bcr-Abl PTKs. This is consistent with the fact that the K_m values of EEIYEPPY for both c-Abl and Bcr-Abl PTKs were almost identical (Table 2). This suggests that the mode of interaction

between EEIYEOPY and c-Abl or Bcr-Abl is similar even though the intrinsic catalytic activity of Bcr-Abl PTK is significantly higher than that of c-Abl PTK. Work is currently underway in our laboratory to obtain SAR data of the group II basic peptides (Table 1) as substrates for both c-Abl and Bcr-Abl.

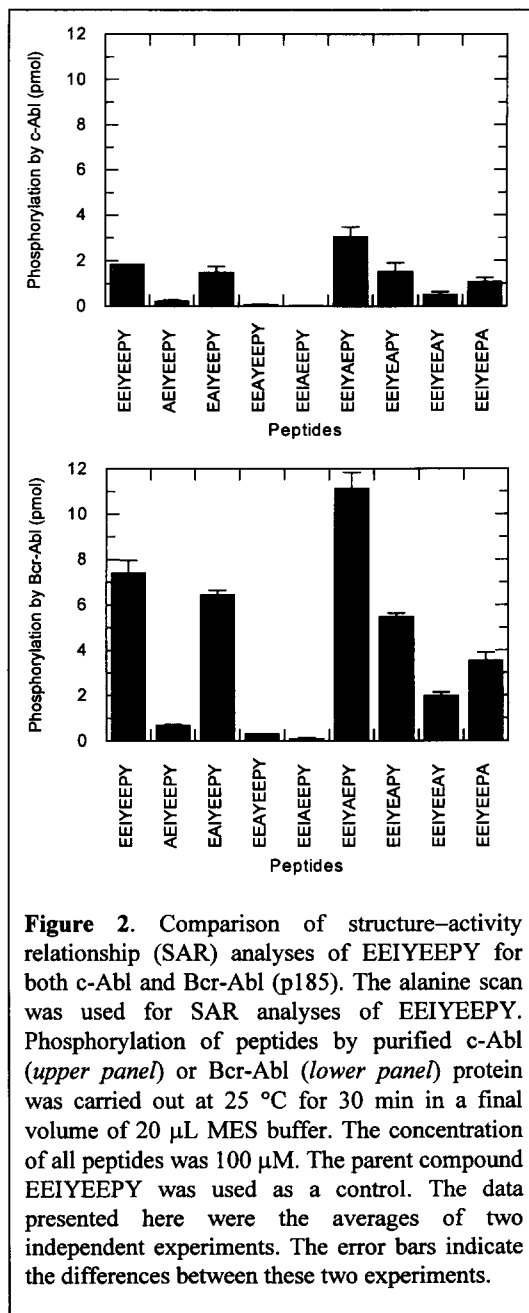
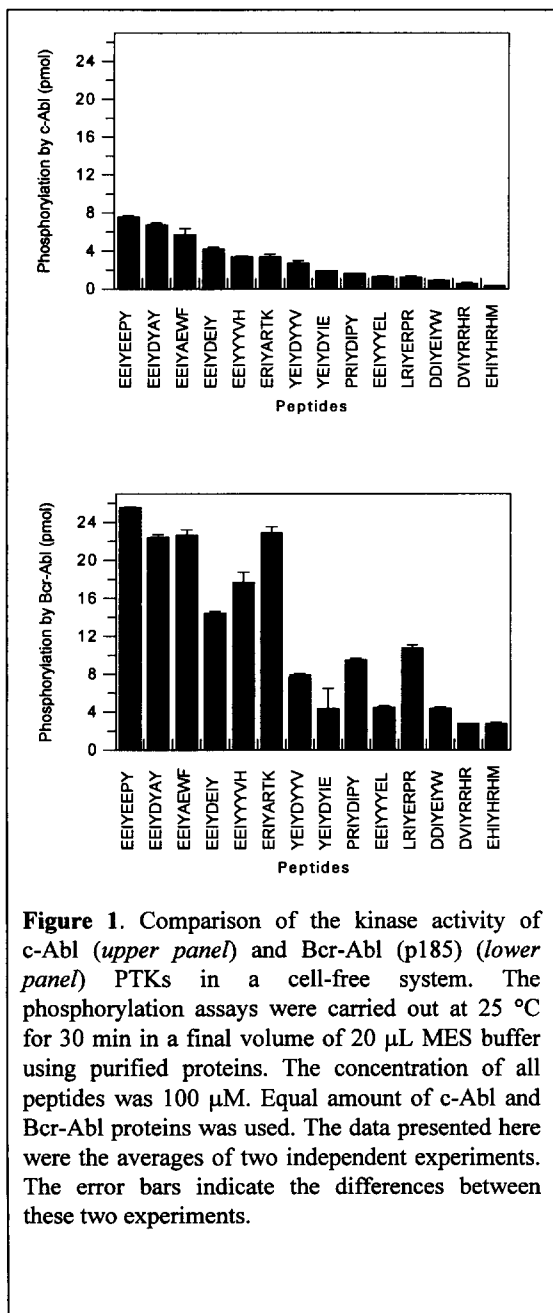


Table 1. Comparison of phosphorylation of peptides by c-Abl and Bcr-Abl PTKs^a

| | Peptide | c-Abl, pmol | Bcr-Abl, pmol | Ratio ^b (Bcr-Abl/c-Abl) |
|----------|----------|-------------|---------------|------------------------------------|
| Group I | YEIYDYIE | 1.89 ± 0.01 | 4.33 ± 2.12 | 2.29 |
| | YEIYDYYV | 2.71 ± 0.26 | 7.89 ± 0.13 | 2.91 |
| | EEIYDYAY | 6.73 ± 0.19 | 22.40 ± 0.30 | 3.33 |
| | EEIYEOPY | 7.56 ± 0.12 | 25.53 ± 0.08 | 3.38 |
| | EEIYDEIY | 4.18 ± 0.20 | 14.41 ± 0.21 | 3.44 |
| | EEIYYYEL | 1.27 ± 0.08 | 4.48 ± 0.15 | 3.52 |
| | EEIYAEWF | 5.69 ± 0.63 | 22.61 ± 0.60 | 3.98 |
| Group II | DDIYEIYW | 0.88 ± 0.05 | 4.35 ± 0.16 | 4.92 |
| | DVIYRRHR | 0.54 ± 0.12 | 2.75 ± 0.01 | 5.06 |
| | EEIYYYVH | 3.37 ± 0.12 | 17.67 ± 1.08 | 5.24 |
| | PRIYDIPY | 1.59 ± 0.04 | 9.50 ± 0.17 | 5.97 |
| | ERIYARTK | 3.37 ± 0.27 | 22.86 ± 0.64 | 6.79 |
| | EHIYHRHM | 0.33 ± 0.02 | 2.73 ± 0.12 | 8.27 |
| | LRIYERPR | 1.23 ± 0.16 | 10.74 ± 0.34 | 8.73 |

^aThe concentration of all peptides was 100 μM, and the phosphorylation assays were carried out at 25 °C for 30 min in a cell-free system. Both c-Abl and Bcr-Abl PTKs were purified from Sf9 cells transfected using a baculovirus system. The same concentration of c-Abl and Bcr-Abl proteins was used in the assays.

^bIndicates ratios of phosphorylation by Bcr-Abl PTK to that by c-Abl PTK for different peptide substrates identified from the combinatorial peptide library.

Table 2. Comparison of kinetic parameters of peptide phosphorylation by c-Abl and Bcr-Abl PTKs^a

| Peptide | K _m , μM | | V _{max} ^b , pmol/min | | V _{max} /K _m , L × 10 ⁻³ /min | |
|----------|---------------------|----------|--|--------------|--|---------|
| | c-Abl | Bcr-Abl | c-Abl | Bcr-Abl | c-Abl | Bcr-Abl |
| EEIYEOPY | 105 ± 13 | 106 ± 10 | 1.28 ± 0.04 | 10.47 ± 0.63 | 12.1 | 98.8 |
| EEIYDYAY | 134 ± 16 | 130 ± 44 | 1.00 ± 0.04 | 7.33 ± 0.83 | 7.46 | 56.4 |
| EEIYAEWF | 51 ± 8 | 54 ± 9 | 0.71 ± 0.03 | 5.27 ± 0.27 | 14.0 | 97.6 |
| EEIYDEIY | 194 ± 52 | 439 ± 67 | 0.73 ± 0.08 | 7.50 ± 0.85 | 3.75 | 17.1 |
| EEIYYYVH | 147 ± 49 | 141 ± 23 | 0.35 ± 0.04 | 4.64 ± 0.26 | 2.38 | 32.9 |
| ERIYARTK | 269 ± 43 | 121 ± 11 | 0.66 ± 0.05 | 5.10 ± 0.18 | 2.47 | 42.2 |

^aBoth c-Abl and Bcr-Abl were purified from Sf9 cells transfected using a baculovirus system.

^bThe data were normalized by the concentration of the enzymes.

Conclusions

We have compared the intrinsic tyrosine kinase activities between c-Abl and Bcr-Abl. Our current data support the hypothesis that the Bcr sequence directly activates the tyrosine kinase activity of Abl. This activation could be achieved intramolecularly or intermolecularly through a conformational change induced by the Bcr fusion. Our results also suggest that the peptide substrate preference of Abl may also be altered to some extent by the fusion of the Bcr sequence to N-terminus of Abl protein. These data may provide some new insights for the mechanism of action of c-Abl and Bcr-Abl PTKs.

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