[CANCER RESEARCH 56, 5120–5124, November 15, 1996]

Advances in Brief

Sequences within the First Exon of *BCR* Inhibit the Activated Tyrosine Kinases of c-Abl and the Bcr-Abl Oncoprotein[1]

Jiaxin Liu,[2] Yun Wu,[2] and Ralph B. Arlinghaus[3]

Department of Molecular Pathology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

Abstract

The Bcr-Abl oncoprotein is the primary causative factor in Philadelphia chromosome-associated leukemias. The activated tyrosine kinase of the Bcr-Abl oncoprotein is the primary driving force behind its oncogenic activity. We report here that a deleted form of Bcr [Bcr(64–413)], encompassing the Abl SH2 binding domains of Bcr, reduced the phosphotyrosine content of c-Abl and Bcr-Abl within cells and inhibited Bcr-Abl autophosphorylation activity *in vitro*. Similarly, a Bcr peptide phosphorylated on Ser-354 blocked the c-Abl and Bcr-Abl kinases *in vitro*, whereas the same peptide phosphorylated on Tyr-360 was not inhibitory. Bcr(64–413) was also resistant to tyrosine phosphorylation by either activated c-Abl or Bcr-Abl. Importantly, Bcr(64–413) interfered with the growth of Bcr-Abl-expressing cell lines. Our findings indicate that the Abl SH2 binding domain of Bcr in the phosphoserine form inhibits the Bcr-Abl oncoprotein but that tyrosine phosphorylation of this domain of Bcr reverses its inhibitory effects on Bcr-Abl. These results raise interesting questions about a possible role of Bcr or a Bcr-related molecule in modulating the activity of the Bcr-Abl oncoprotein and c-Abl itself.

Introduction

Fusion of Bcr sequences to Abl sequences results in activation of the Abl tyrosine kinase domain (1, 2), which is the principal driving force for the leukemic phenotype (3, 4). The activity of the Abl tyrosine kinase domain is regulated by the SH3[4] and SH2 regions. Deletions of the SH3 domain activate the Abl kinase (5), whereas alterations in the SH2 domain decrease the Abl kinase activity (6). The Bcr protein forms a complex with Bcr-Abl (7) and is phosphorylated by Bcr-Abl (8), and the sites of Bcr tyrosine phosphorylation are similar to those found in Bcr-Abl (9, 10).

The first exon of Bcr encodes a novel serine/threonine kinase (11). Within this coding region are two domains termed A and B boxes, which bind to the Abl SH2 domain in a non-phosphotyrosine-dependent manner (12). We report here that a deleted form of a Bcr sequence containing these A and B box sequences, when constructed in a way that makes it resistant to tyrosine phosphorylation by activated Abl, inhibited the activated c-Abl and Bcr-Abl tyrosine kinases.

Materials and Methods

Cells and Antibodies. COS-1, SUP B15, and K562 cells were maintained as described previously (8, 9). Anti-Abl 8E9 and anti-Abl(51–64) are monoclonal antibodies against two different regions of c-Abl (8–10). Anti-Bcr(181–194) is a rabbit antipeptide antiserum raised against a synthetic peptide encompassing the 181–194 amino acid sequence within the first exon of Bcr. PY20 is an antiphosphotyrosine antibody purchased from Transduction Laboratories, Inc. (Lexington, KY). Western blotting was performed by the ECL method using commercial reagents from Amersham Corp. (Arlington Heights, IL).

Bcr Plasmid and Peptides. The DNA sequence encoding Bcr(64–413) was derived from a Bcr-Abl DNA clone provided by Jean Wang (University of California, San Diego) (13). The Bcr sequence was inserted into the *Bam*HI site of pLNL SLX cytomegalovirus vector (13) using a linker sequence. The translation product would begin with the amino acid sequence MAAAK fused to the amino-terminal Bcr sequence beginning with amino acid 64 (beginning with the sequence AKE); the linker sequence at the 3′ end added amino acids L and V to the carboxyl terminus of this Bcr sequence at amino acid 413 (ending with the sequence GQI) followed by a stop codon. The serine-rich A box includes residues 197–239; the B box includes residues 299–385.

Synthetic peptides were prepared in our Synthetic Antigen Facility. Peptides were purified to greater than 95% by high-performance liquid chromatography methods. The presence of phosphorylated residues was verified by mass spectrometry analysis.

DNA Transfection. COS-1 cells were transiently transfected with a pSG5 plasmid (Stratagene, La Jolla, CA) containing c-ABL (8) or cotransfected with c-ABL and either BCR(64–413) or BCR(1–413) in the same vector. Extracts were divided into equal portions for analysis by Western blotting with anti-Abl 8E9 monoclonal antibody, antiphosphotyrosine antibody (PY20; Transduction Laboratories, Inc., Lexington, KY), and anti-Bcr antibody (181–194). For stable transfection, BCR(64–413) was inserted into the pLNL SLX vector (13) downstream of a cytomegalovirus promoter. This vector contains a *neo* resistance gene. Transfected K562 cells were seeded in soft agar in the presence of 400 μg/ml geneticin. Colonies were counted at two weeks of selection.

Immunoprecipitation and Kinase Assays. Kinase assays were performed as described (10). Immune complexes harvested from COS-1 cells expressing Bcr(64–413) were obtained by immunoprecipitation with anti-Bcr(181–194) followed by binding to protein A-Sepharose beads. Bcr-Abl or c-Abl immune complexes were obtained from either SUP B15 cells (P185 BCR-ABL) or COS-1 cells (c-Abl) by immunoprecipitation with anti-Abl(51–64) (termed p6D). Peptides were preincubated with Abl or Bcr-Abl immune complexes on ice for 5 min prior to the addition of the kinase reaction buffer containing the labeled ATP. Proteins were analyzed on SDS gels as described (10). Use of purified antibody is required to observe the inhibitory effect of the p354 S17K peptide. S17K has the sequence 350-SSRVSPSPTTYRMFRDK-366. Assays involving c-Src were performed as described (14).

Results and Discussion

Because the first exon of Bcr is known to bind to the Abl SH2 domain in a non-phosphotyrosine-dependent manner (12) and the fact that Bcr first exon sequences are a target for both Bcr-Abl (9, 10) and activated c-Abl tyrosine kinases,[5] we determined whether a nonphosphotyrosine form of Bcr first exon sequences would stimulate or inhibit the activated c-Abl tyrosine kinase. Two different constructs of Bcr first exon sequences were separately expressed in COS-1 cells together with c-Abl under conditions that activate the Abl tyrosine kinase (Ref. 15; Fig. 1). One construct [Bcr(64–413)] lacked the Bcr coiled-coil oligomerization domain, which is responsible for activating the Abl kinase domain of Bcr-Abl (13). The other construct [Bcr(1–413)] encoded Bcr residues 1–413 within the 426-amino acid first exon. Bcr(1–413) was an excellent target for the c-Abl tyrosine

Received 8/14/96; accepted 10/1/96.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by grants from the NIH (CA65611 and CA16672). R. B. A. holds the Stringer chair of Cancer Research.

[2] The first two authors contributed equally to this work.

[3] To whom requests for reprints should be addressed.

[4] The abbreviations used are: SH, Src homology; ECL, enhanced chemiluminescence.

[5] Unpublished data.

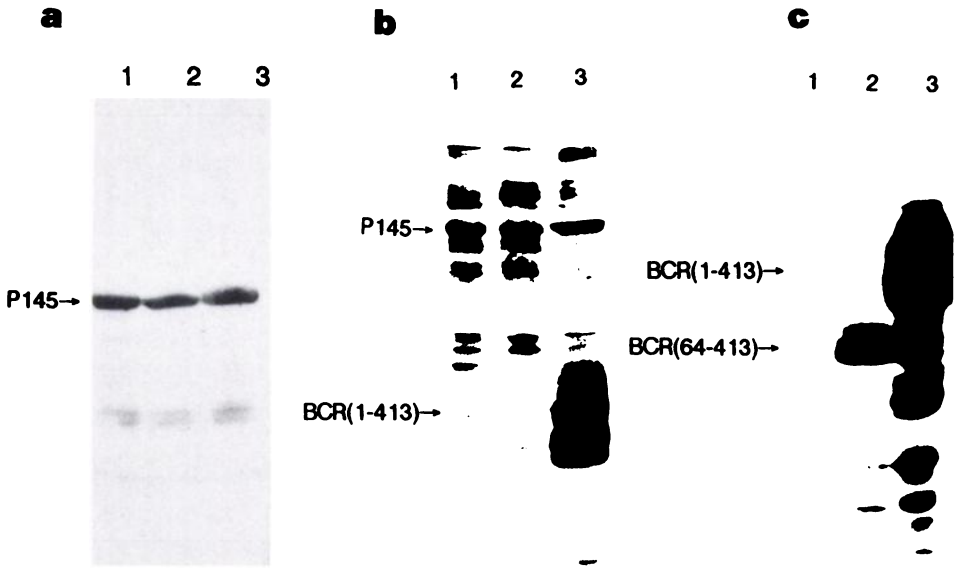

Fig. 1. Bcr sequences lacking the oligomerization domain are not a target for activated Abl and inhibit tyrosine phosphorylation of c-Abl. *a*, Western blotting detection of P145 ABL in COS-1 cells. *Lane 1*, c-Abl expression alone; *Lane 2*, coexpression of c-Abl and Bcr(64–413); *Lane 3*, coexpression of c-Abl and Bcr(1–413). *b*, Antiphosphotyrosine blotting of COS-1 cells coexpressing c-Abl and either Bcr(64–413) or Bcr(1–413). *Lanes 1, 2,* and *3* are the same extracts described in *a*. *c*, Western blotting detection of Bcr(64–413) and Bcr(1–413) by anti-Bcr(181–194). *Lanes 1, 2,* and *3* are the same as in *a* except that the bottom half of the gel was used for blotting by Western blotting with anti-Bcr(181–194). Western blotting was carried out using standard ECL procedures. The intensity of bands in *b* was determined by scanning with a volume densitometer (Personal Densitometer, Molecular Dynamics, Sunnyvale, CA).

kinase (Fig. 1*b*, *Lane 3*), whereas Bcr(64–413) was not (Fig. 1*b*, *Lane 2*). Bcr(64–413) should not oligomerize, whereas Bcr(1–413) should form the homotetramer Bcr structure (13). Both proteins were expressed at high levels as determined by Western blotting of the same extracts (Fig. 1*c*, *Lanes 2* and *3*). Of importance, coexpression by transient transfection of Bcr(64–413) with c-Abl inhibited the tyrosine phosphorylation of P145 Abl by about 50% (Fig. 1*b*, *Lane 2*), whereas coexpressing Bcr(1–413) with c-Abl had no effect on c-Abl tyrosine phosphorylation (Fig. 1*b*, *Lane 3*). These results raise the possibility that Bcr, which is not tyrosine phosphorylated, is inhibitory to the c-Abl tyrosine kinase, but tyrosine-phosphorylated Bcr lacks this inhibitory activity. The inhibitory effects of Bcr(64–413) are understated undoubtedly because of the difficulty in transient transfection of having each cell express both proteins. The same extracts were shown to have similar levels of c-Abl by Western blotting with anti-Abl antibody (Fig. 1*a*).

In other experiments, Bcr(64–413) was transfected into K562 cells under conditions of G418 selection to determine its effect on Bcr-Abl tyrosine phosphorylation. Clones that expressed Bcr(64–413) had a reduced level of phosphotyrosine-containing P210 BCR-ABL relative to K562 cells transfected with the vector alone (results not shown).

Because non-phosphotyrosine-containing Bcr(64–413) inhibited tyrosine phosphorylation of activated c-Abl and Bcr-Abl in transfected cells, we tested the growth-inhibitory effects of Bcr(64–413) in a Bcr-Abl-expressing cell line derived from a Philadelphia chromosome-positive leukemia patient (K562 cells). In these experiments, the vector either lacking Bcr sequences or encoding Bcr(64–413) was transfected into K562 cells (which express P210 Bcr-Abl) under conditions for G418 selection, and cells were selected for growth in soft agar for 2 weeks (Fig. 2). In this experiment, colony formation was reduced 70% by expression of Bcr(64–413) compared to vector only. In a second experiment, colony formation was reduced by about 50% (Table 1). Several soft agar colonies of K562 cells transfected with Bcr(64–413) DNA lacked detectable Bcr(64–413), whereas others had low level expression of Bcr(64–413) (data not shown). Similar reduction of colony formation was obtained with Bcr(64–413)-expressing KBM-7 cells, which also express P210 Bcr-Abl (data not shown).

To determine whether Bcr(64–413) could directly inhibit the tyrosine kinase activity of Bcr-Abl, we immunoprecipitated Bcr(64–413) from COS-1 cells (as in Fig. 1*c*, *Lane 2*) with anti-Bcr(181–194) and mixed the immunoprecipitate with P185 BCR-ABL harvested with

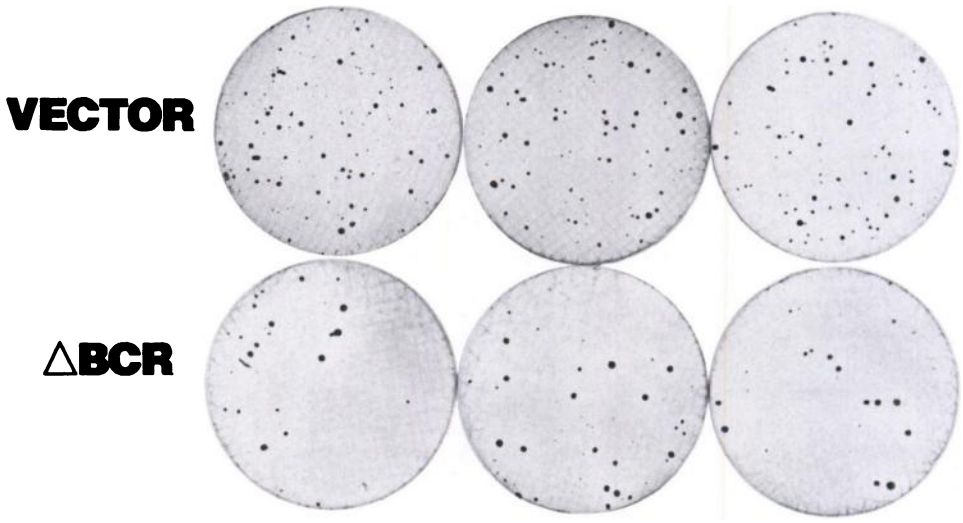

Fig. 2. Bcr(64–413) decreases the colony formation capacity of Bcr-Abl-expressing leukemia cells. K562 cells were transfected with DNA encoding Bcr(64–413) (Δ*Bcr*) or vector DNA only.

Table 1 *BCR(64–413) expression decreases colony formation capacity of BCR-ABL leukemia cells*

K562 cells were electrophoresed with 30 μg DNA. After 48 h 5×10^5 cells were plated in soft agar with 400 μg/ml G418 per 6-cm dish. Colonies were counted at 2 weeks.

Construct	Colony numbers per 10^6 cells[a]	
	assay 1	assay 2
pLNL SLX	230 ± 10	127 ± 12
pLNL SLX Bcr(64–413)	128 ± 4	43 ± 7

[a] Colony numbers are the average of three plates per construct.

anti-Abl(51–64) from SUP-B15 leukemic cells (Fig. 3*a*). The results showed that P185 autokinase activity was dramatically inhibited (*Lane 2*) compared to untreated (*Lane 4*) or antibody treatment only (Fig. 3*c*, *Lane 2, top portion*). In contrast, a boiled Bcr(64–413) immunoprecipitate had greatly reduced inhibitory activity (Fig. 3*a*, *Lane 1*). These results demonstrate that Bcr(64–413) directly inhibits Bcr-Abl tyrosine kinase activity. In similar types of experiments, Bcr(1–413) did not inhibit the Bcr-Abl or Abl kinases (data not shown) because it was an excellent substrate for these tyrosine kinases, in agreement with our previous studies (9, 10).

Because of its Abl SH2 binding domains, Bcr(64–413) should bind to the SH2 domain of c-Abl or Bcr-Abl and form stable complexes. Such complexes should be significantly less kinase active than control Abl proteins. Therefore, we tested the *in vitro* kinase activity of c-Abl and Bcr-Abl isolated from cells expressing Bcr(64–413). Extracts were made from COS-1 cells expressing Bcr(64–413) and P145 ABL, and the Abl-containing proteins were immunoprecipitated with anti-Abl(51–64) monoclonal antibody and kinase assays performed (Fig. 3*d*). P145 ABL kinase from cells expressing both P145 ABL and Bcr(64–413) was inhibited by more than 95% (*Lane 2*) compared to COS-1 cells expressing only P145 ABL (*Lane 1*). Western blotting with anti-Abl 8E9 monoclonal antibody showed little change in the concentration of P145 ABL in cells coexpressing Bcr(64–413) (Fig. 3*e*). Similar effects were obtained with P210 BCR-ABL (data not shown). Considering the previous data of Pendergast *et al.* (12), these findings indicate that the inhibitory effects of Bcr(64–413) result from a complex between P145 ABL and Bcr(64–413). In support of this conclusion, Bcr(64–413) was shown to bind to GST-Abl SH2 sequences (not shown).

To investigate the regions of Bcr(64–413) that are responsible for the kinase-inhibitory effects, we made a series of synthetic peptides from this coding region of Bcr. Our results showed that a 17-amino acid Bcr peptide (S17K) from the B box region of Bcr (12), which was phosphorylated on Ser-354 (p354), strongly inhibited both the Bcr-Abl kinase (Fig. 4*a*, *Lanes 2* and *6*) and the c-Abl kinase (*Lane 4*) whereas the unphosphorylated form of the peptide was not inhibitory (Fig. 3*a*, *Lane 3*). Of interest, S17K phosphorylated on Tyr-360 was also not inhibitory to the c-Abl or Bcr-Abl kinases (not shown). The inhibitory effect of (pSer-354 S17K) was concentration dependent as shown in Fig. 4*b*. Neither S17K nor pSer-354 S17K inhibited the c-Src kinase (Fig. 4*c*).

The results reported here provide strong evidence that non-tyrosine-phosphorylated sequences within the first exon of Bcr inhibit the activated c-Abl protein and the Bcr-Abl oncoprotein. Our findings indicate that serine-phosphorylated first exon Bcr sequences, and not the tyrosine-phosphorylated sequences, are the functional inhibitor. Although we do not know the precise mechanism by which serine-phosphorylated Bcr inhibits the activated Abl tyrosine kinase, the earlier findings by Pendergast *et al.* (12) provide a basis for this inhibitory activity. In their studies, two regions encoded by the first Bcr exon bound tightly to Abl SH2 sequences in a non-phosphotyrosine-dependent manner (12). These regions were termed the A and B boxes; both of them are serine-rich. Bcr(64–413) contains both of these serine-rich boxes. Because A and B boxes were required for fibroblast transformation by Bcr-Abl, Pendergast *et al.* (12) speculated that A and B box Bcr sequences were responsible for activating

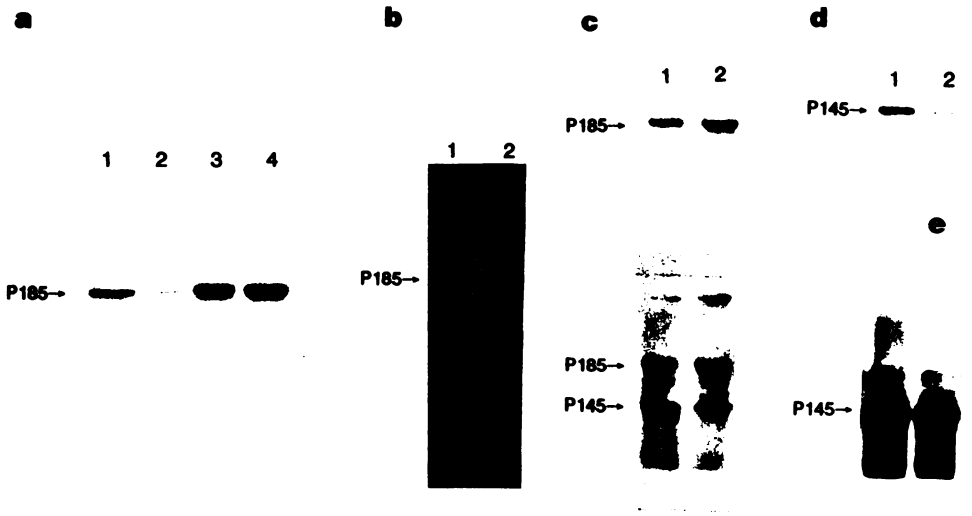

Fig. 3. Bcr(64–413) inhibits Bcr-Abl tyrosine kinase activity *in vitro*. Bcr(64–413) isolated from COS-1 cells was mixed with P185 BCR-ABL from B15 cells to test its effects on the Bcr-Abl tyrosine kinase activity. *a*, effect of Bcr(64–413) on Bcr-Abl kinase activity *in vitro*. *Lane 1*, boiled anti-Bcr immune complexes containing Bcr(64–413) from COS-1 cells were added to P185 BCR-ABL immune complexes from SUP B15 cells; *Lane 2*, nondenatured immune complexes of Bcr(64–413) mixed with P185 BCR-ABL; *Lane 3*, 100 μg of Bcr peptide S17K (not phosphorylated) added to P185 BCR-ABL immune complexes; *Lane 4*, P185 BCR-ABL immune complexes alone. *b*, Western blotting of the immunoprecipitates of P185 BCR-ABL with anti-Bcr(181–194). *Lane 1*, Western blotting of immune complexes using anti-Bcr(181–194) from the reaction mixture shown in *Lane 2* of *a*; *Lane 2*, anti-Bcr(181–194) Western blotting of immune complexes from *Lane 4* of *a*. *c*, Anti-Bcr(181–194) itself does not block P185 BCR-ABL kinase activity. *Lane 1*, P185 BCR-ABL immune complexes alone; *Lane 2*, anti-Bcr(181–194) protein A-Sepharose beads lacking Bcr(64–413) mixed with P185 BCR-ABL complexes. The *bottom* portion of *c* shows the Western blotting of P185 BCR-ABL immunoprecipitates with anti-Abl 8E9 after blotting onto a membrane using the gel shown in the *top* portion of *c*. *Lanes 1* and *2* in the *bottom* portion of *c* correspond to *Lanes 1* and *2* in the *top* portion of *c*, as a result of probing with anti-Abl 8E9 monoclonal antibody. *d*, coexpression of Bcr(64–413) and c-Abl inhibits P145 ABL tyrosine kinase activity. *Lane 1*, tyrosine kinase activity of P145 ABL expressed in COS-1 cells lacking Bcr(64–413); *Lane 2*, kinase activity of P145 ABL extracted from COS-1 cells expressing both P145 ABL and Bcr(64–413). *e*, Western blotting of COS-1 cells expressing P145 ABL in the presence (*Lane 2*) and absence (*Lane 1*) of Bcr(64–413). The gel used in *d* was electroblotted, and the membrane was probed with anti-Abl 8E9 using ECL. The intensity of the P145 Abl bands in *d* was determined by a volume densitometer (Personal Densitometer, Molecular Dynamics). In *c* and *d*, gels were first exposed to X-ray film and then electroblotted onto membrane for probing with antibody using ECL procedures.

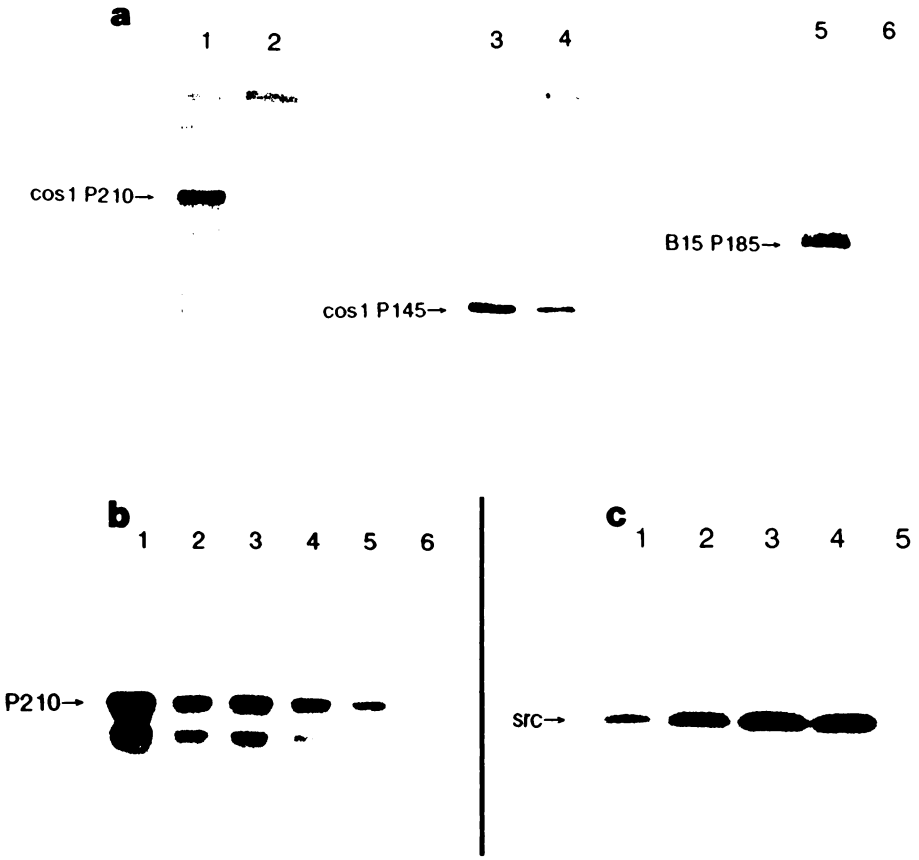

Fig. 4. A phosphoserine peptide from the B box region of Bcr inhibits Abl and Bcr-Abl tyrosine kinase activity *in vitro*. *a*, Bcr peptide p354S S17K inhibits Abl and Bcr-Abl tyrosine kinase activity *in vitro*. *Lane 1*, P210 BCR-ABL kinase activity in absence of peptide; *Lane 2*, P210 kinase activity after treatment with 100 μg of p354S S17K; *Lane 3*, P145 ABL; *Lane 4*, P145 ABL plus 100 μg of p354S S17K; *Lane 5*, P185 BCR-ABL; *Lane 6*, P185 plus 100 μg of p354S S17K. *b*, Dose-dependent inhibition of P210 BCR-ABL by p354S S17K. *Lane 1*, P210 BCR-ABL alone; *Lane 2*, P210 plus 10 μg of p354S S17K; *Lane 3*, P210 plus 20 μg of peptide; *Lane 4*, P210 plus 40 μg of peptide; *Lane 5*, P210 plus 80 μg of peptide; *Lane 6*, P210 plus 160 μg of peptide. *c*, p354S S17K does not inhibit the c-Src kinase. *Lane 1*, p60 c-Src immune complexes in Src kinase reaction buffer (containing MgCl2; Ref. 14); *Lane 2*, c-Src immune complexes in our Abl kinase reaction buffer (in $MnCl_2$; Ref. 10); *Lane 3*, s-Src immune complexes plus 100 μg of p354S S17K Bcr peptide in the MnCl2 reaction buffer; *Lane 4*, c-Src immune complexes plus 100 μg of S17K Bcr peptide; *Lane 5*, c-Src plus 75 ng of Herbamycin, a known inhibitor of c-Src (14). Tyrosine kinase assays were performed as described (10).

the Abl kinase of Bcr-Abl. Our findings indicate that these Bcr sequences are not activators of the Bcr-Abl tyrosine kinase but are inhibitory when in serine-phosphorylated form. Subsequently, it was discovered that phosphotyrosine 177, just upstream of the A box Bcr sequences, plays a major role in Bcr-Abl transformation effects by activating the Ras pathway (16, 17).

Our experimental results have shown that Bcr(64–413) expressed in COS-1 cells binds to GST-Abl SH2 sequences *in vitro* (results not shown). Moreover, coexpression of Bcr(64–413) with P145 ABL indicated that the inhibitory effects of Bcr(64–413) result from a complex between the two proteins, as isolation of P145 ABL from Bcr(64–413)-expressing cells resulted in more than 95% inhibition of the c-Abl tyrosine kinase (Fig. 3*d*). In this regard, Pendergast *et al.* (12) demonstrated that P160 BCR and P145 ABL coprecipitate when coexpressed in insect cells. We also identified a 17-amino acid phosphopeptide (pSer-354 S17K) from the B box region of Bcr that inhibited the Bcr-Abl and c-Abl tyrosine kinases (Fig. 4, *a* and *b*). Of importance, this peptide did not inhibit the c-Src tyrosine kinase (Fig. 4*c*), indicating that the inhibitory effect may be specific to the Abl family of tyrosine kinases. On the basis of these findings, we conclude that these first exon Bcr sequences, when phosphorylated on serine residues, bind to the Abl SH2 domain, resulting in the inactivation of the Abl tyrosine kinase activity. In contrast, when these Bcr sequences are tyrosine-phosphorylated, their inhibitory activity is lost (Fig. 1).

Studies are under way to determine whether Bcr itself can antagonize Bcr-Abl transforming activity. Our previous work (8) demonstrated that tyrosine phosphorylation of Bcr-Abl (*i.e.*, its autokinase activity) is not inhibited by coexpression of P160 BCR. This lack of inhibition is explained by its tyrosine phosphorylation by Bcr-Abl, as was the case with Bcr(1–413) and c-Abl coexpression, shown in Fig. 1. Thus, we would argue that tyrosine-phosphorylated P160 BCR is neutralized in its Abl kinase-inhibitory effects, converting P160 BCR into an oncogenic effector through its phosphorylation on tyrosine 177 (16).[6] Our previous studies have also established that tyrosine phosphorylation of Bcr inhibits its serine/threonine kinase activity (10). It remains to be demonstrated whether a monomeric form of Bcr or yet another form of Bcr antagonizes Bcr-Abl and c-Abl tyrosine kinases. Nevertheless, our findings raise the possibility that Bcr(64–413) could form the basis of a therapeutic agent for these leukemias, particularly in the chronic phase of CML.

Acknowledgments

We thank Jean Wang's laboratory (University of California, San Diego) for providing a Bcr-Abl DNA clone that lacks the first 63 amino acids of Bcr. We also thank Dr. Gary Gallick for performing the c-Src kinase assays.

References

1. Konopka, J. B., Watanabe, S. M., and Witte, O. N. An alteration of the human *c-abl* protein in K562 leukemia cells unmasks associated tyrosine kinase activity. Cell, *37:* 1035–1042, 1984.
2. Kloetzer, W., Kurzrock, R., Smith, L., Talpaz, M., Spiller, M., Gutterman, J., and Arlinghaus, R. B. The human cellular *abl* gene product in the chronic myelogenous leukemia cell line K562 has an associated tyrosine protein kinase activity. Virology, *110:* 230–238, 1985.
3. Daley, G. Q., Van Etten, R. A., and Baltimore, D. Induction of chronic myelogenous leukemia in mice by the $P210^{bcr/abl}$ gene of the Philadelphia chromosome. Science (Washington DC), *247:* 824–830, 1990.
4. Heisterkamp, N., Jenster, G., ten Hoeve, J., Zovich, D., Pattengale, P. K., and Groffen, J. Acute leukemia in BCR-ABL transgenic mice. Nature (Lond.), *344:* 251–253, 1990.
5. Franz, W. M., Berger, P., and Wang, J. Y. J. Deletion of an N-terminal regulatory domain of the c-abl tyrosine kinase activates its oncogenic potential. EMBO J., *8:* 137–147, 1989.
6. Mayer, B. J., Jackson, P. K., VanEtten, R. A., and Baltimore, D. Point mutations in the Abl SH2 domain coordinately impair phosphotyrosine binding *in vitro* and transforming activity *in vivo*. Mol. Cell. Biol., *12:* 609–618, 1992.

[6] G. Ma, D. Lu, J. Liu, Y. Wu, and R. B. Arlinghaus. Bcr phosphorylated on tyrosine 177 binds Grb2, manuscript in preparation.

7. Campbell, M., Li, W., and Arlinghaus, R. B. P210 BCR-ABL is complexed to P160 BCR and ph-P53 proteins in K562 cells. Oncogene, *5:* 773–776, 1990.
8. Lu, D., Liu, J., Campbell, M., Guo, J. Q., Heisterkamp, N., Groffen, J., Canaani, E., and Arlinghaus, R. B. Tyrosine phosphorylation of P160 BCR by P210 BCR-ABL. Blood, *82:* 1257–1263, 1993.
9. Liu, J., Campbell, M., Guo, J. Q., Lu, D., Xian, Y. M., and Arlinghaus, R. B. BCR-ABL tyrosine kinase autophosphorylates itself or transphosphorylates P160 BCR on tyrosine predominantly within the first exon. Oncogene, *8:* 101–109, 1993.
10. Liu, J., Wu, Y., Ma, G., Lu, D., Haataja, L., Heisterkamp, N., Groffen, J., and Arlinghaus, R. B. Inhibition of Bcr serine kinase by tyrosine phosphorylation. Mol. Cell. Biol., *16:* 998–1005, 1996.
11. Maru, Y., and Witte, O. N. The *BCR* gene encodes a novel serine/threonine kinase activity within a single exon. Cell, *67:* 459–468, 1991.
12. Pendergast, A. M., Muller, A. J., Havlik, M. H., Maru, Y., and Witte, O. N. BCR sequences essential for transformation by the *BCR-ABL* oncogene bind to the ABL SH2 regulatory domain in a non-phosphotyrosine-dependent manner. Cell, *66:* 161–171, 1991.
13. McWhirter, J. R., Galasso, D. L., and Wang, J. Y. J. A coiled-coil oligomerization domain of Bcr is essential for the transforming function of Bcr-Abl oncoproteins. Mol. Cell. Biol., *13:* 7587–7595, 1993.
14. Garcia, R. A., Saya, H., and Gallick, G. E. Effect of Herbamycin A on growth and pp60 cc-Src activity in human colon tumor cell lines. Oncogene, *6:* 1983–1989, 1991.
15. Pendergast, A. M., Muller, A. J., Havlik, M. H., Clark, R., McCormick, F., and Witte, O. N. Evidence for regulation of the ABL tyrosine kinase by a cellular inhibitor. Proc. Natl. Acad. Sci. USA., *88:* 5927–5931, 1991.
16. Puil, L., Liu, J., Gish, G., Mbalamu, G., Arlinghaus, R., Pelicci, P. G., and Pawson, T. BCR-ABL oncoproteins bind directly to activators of the Ras signalling pathway. EMBO J., *13:* 764–773, 1994.
17. Pendergast, A. M., Quilliam, L. A., Cripe, L. D., Bassing, C. H., Dai, Z., Li, N., Batzer, A., Rabun, K. M., Der, C. J., Schlessinger, J., and Gishizky, M. L. BCR-ABL-induced oncogenesis is mediated by direct interaction with the SH2 domain of the GRB-2 adaptor protein. Cell, *75:* 175–185, 1993.