Interaction of Nucleolar Protein B23 with Peptides Related to Nuclear Localization Signals†

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ABSTRACT: Nucleolar protein B23 is a putative ribosome assembly factor with a high affinity for peptides containing nuclear localization signals (NLSs). The interactions of various NLS-containing peptides with two B23 isoforms (B23.1 and B23.2) were examined using equilibrium dialysis and Scatchard analyses. The K_D for protein B23 binding to a peptide containing the SV40 T-antigen NLS sequence was approximately 1 μM with a stoichiometry of 1:1 (peptide:protein). No significant differences were seen between the two B23 isoforms in their affinities for any of the peptides tested. Binding by a reverse sequence SV40 T-NLS peptide showed a nonlinear Scatchard plot: this peptide was unable displace the correct sequence peptide, suggesting that the reverse sequence peptide binds to a different site on the protein. A peptide containing the sequence required for nucleolar localization of the HIV-1 Rev protein had an affinity for B23 approximately 10-fold greater than that of the SV40 T-NLS. However, with a sequence sufficient only for Rev location in the nucleoplasm, the affinity for B23 was diminished to a level between that of the longer Rev sequence and the SV40 T-NLS. In competition binding assays, the Rev NLS peptide was able to displace the SV40 T NLS, indicating that both peptides bind to the same site on protein B23. There was no detectable binding to protein B23 by a peptide containing the bipartite NLS of nucleoplasmin. Phosphorylation of protein B23 by casein kinase II enhanced its affinity for the SV40 T- and Rev-derived peptides approximately 2-fold. This effect was not seen with cdc2 kinase-phosphorylated B23. These data support the idea that protein B23 is a carrier and/or nucleolar receptor of proteins bearing NLS sequences of the SV40 T-antigen class and that this interaction may be modulated by phosphorylation.

The import of proteins into the nucleus via the nuclear pore complex is directed by relatively short basic sequences called nuclear localization signals (NLSs)[1] contained in these proteins (Boulikas, 1994; Fabre & Hurt, 1994). The NLS sequences appear to be of 2 classes: those resembling the NLS found in the prototypical SV40 large T antigen (PKKKRKV) in which five of the seven amino acid residues are basic (Kalderon et al., 1984) and those termed bipartite, as typified by the nucleoplasmin NLS (Robbins et al., 1991), where 2 critical basic domains are separated by 10 noncritical amino acid residues.

Although the NLS sequences have been identified in a large number of proteins, only a few of the protein receptors to which they bind have been identified. One of these is nucleolar protein B23, which was shown to bind peptides containing the NLS from the SV40 T antigen (Goldfarb, 1988). Protein B23 (also called nucleophosmin, numatrin, or NO38) is an abundant multifunctional phosphoprotein also predominantly located in the nucleolus. A nuclear import and/or receptor function for protein B23 was suggested initially when it was discovered that it contains highly acidic segments (Mamrack et al., 1977) which should be capable of binding basic sequences of ribosomal proteins (Olson, 1990). Protein B23 has also been shown to shuttle between the nucleolus and cytoplasm (Borer et al., 1989). Furthermore, protein B23 forms a specific complex with the human immunodeficiency virus-1 (HIV-1) Rev protein (Fankhauser et al., 1991; Umekawa et al., 1993). Taken together, the above data suggest a role for protein B23 in nuclear or nucleolar protein import.

Protein B23 is expressed in at least two isoforms (Chan et al., 1985, 1986). Chang at al. (1988, 1989) characterized two full-length cDNAs for rat protein B23 which contain identical 5′ regions and distinct 3′ regions. These code for proteins B23.1 and B23.2 which contain 292 and 257 amino acids, respectively. In their C-terminal sequences beyond the point of identity, there is a unique 37 residue extension in B23.1 and a 2 residue extension in B23.2 (Chang et al., 1990). However, protein B23.1 is found almost exclusively in the nucleolus, and B23.2 appears to be present in the cytoplasm and/or nucleoplasm (Wang et al., 1993). Furthermore, protein B23.1 binds nucleic acids whereas protein B23.2 does not, suggesting that the nucleolar location of the former protein is due to its nucleic acid binding activity which resides in its carboxyl-terminal end (Wang et al., 1994). Since both forms bind the Rev protein (Umekawa et al., 1993), the NLS binding functions appear to be contained somewhere in the common segment of the molecule.

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[1] Abbreviations: NLS, nuclear localization signal; cdc, cell division cycle; CKII, casein kinase II; HIV, human immunodeficiency virus; NEM, *N*-ethylmaleimide; TCA, trichloroacetic acid.

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Phosphorylation of protein B23 has been shown at one or more typical casein kinase II sites (Chan et al., 1986a) and at cdc2-type kinase sites (Jones et al., 1981; Peter et al., 1990). The levels of phosphorylation of the former sites are correlated with the rate of nucleolar synthesis of preribosomal RNA (Olson, 1990), with the latter sites being phosphorylated only during metaphase (Peter et al., 1990). Hence, casein kinase II phosphorylation appears to play a role in ribosome biogenesis whereas the phosphorylation of protein B23 at the cdc2-type sites is believed to be related to the breakdown of the nucleolus during mitosis.

The current studies were undertaken to determine the relative affinities and stoichiometries of various NLS-containing peptides for protein B23 in its unmodified and phosphorylated forms. It was found that protein B23 binds SV40 T-antigen NLS sequences with high affinity and at a 1:1 molar stoichiometry: phosphorylation of the protein by casein kinase II enhances that affinity. In contrast, a reverse sequence SV40 T-antigen NLS appears to bind nonspecifically, and peptides containing a bipartite NLS do not have any appreciable affinity for protein B23. These studies suggest that there is specificity in the interaction of B23 with NLS sequences and that the affinity may be modulated by phosphorylation.

EXPERIMENTAL PROCEDURES

Peptides and Chemicals. Peptides (see Table 1) containing the SV40 large T-antigen NLS sequence (T-NLS), the reverse sequence T-NLS (rNLS), and residues 37−47 of the HIV-1 Rev protein (Rev37−47) were synthesized by Coast Scientific. Peptides based on the nucleoplasmin NLS (npNLS) and residues 40−46 of the Rev protein (Rev40−46) were obtained from Chiron Mimotopes. *N*-[*Ethyl*-2-^{3}H]maleimide ([^{3}H]NEM) was an NEN−Dupont product. Dialysis membranes were obtained from Spectrum Medical Industries, and the equilibrium dialysis cells were made by Scienceware. All of the other chemicals were purchased from Sigma.

Protein B23 Isoforms. Recombinant proteins B23.1 and B23.2 used in these studies were produced in *E. coli* and purified essentially as previously described (Umekawa et al., 1993) except that the respective cDNAs were inserted into the pET 11c vector for expression (Novagen).

Labeling of Peptides. The peptides used for the binding studies were labeled with [^{3}H]NEM through their sulfhydryl groups. The reaction was carried out in 1 mL of phosphate buffer (pH 7.4) which contained 4 μmol of peptide and 2 μmol of mercaptoethanol. This mixture was allowed to stand at room temperature for 2 h, after which 2−10 μCi of [^{3}H]-NEM was added and then incubated overnight at 4 °C. The labeled peptide was separated from the free [^{3}H]NEM by a Sephadex G-10 column. The concentrations of the ^{3}H-labeled peptides were determined by the absorbance at 280 nm. Radioactivity was measured in a liquid scintillation counter using ScintiVerse 30A liquid scintillation cocktail (Fisher Biotech).

Equilibrium Dialysis. Samples of protein B23 (100 nM) and peptides at various concentrations were prepared in dialysis buffer consisting of 20 mM HEPES (pH 7.3) containing 0.5 mM DTT and 0.5 mM EDTA. Dialysis was performed in microdialysis cells (1 mL per chamber) at 25 °C for 6 h. Estimates of K_Ds and stoichiometries of binding were determined by Scatchard analyses (Dahlquist, 1978).

Table 1: Primary Structures of Synthetic Peptides Related to the Nuclear Localization Signal

peptide	sequence
T-NLS	PKKKRKVEDPYC
rNLS	CYPDEVKRKKKP
Rev37−47	ARRNRRRRWREYC
Rev40−46	NRRRRWEYC
npNLS	KRPAATKKAGQAKKKKYC

The binding constants were then determined using FitAll (MTR Software, Toronto, Ontario). The data were fit by nonlinear least-squares analyses using a model that assumes independent noninteracting sites.

Protein Phosphorylation. Recombinant B23 isoforms were phosphorylated *in vitro* with either casein kinase II (Boehringer-Mannheim) or the cdc2 kinase (Upstate Biotechnology) under the reaction conditions described below. For casein kinase II phosphorylation, samples of protein B23.1 or B23.2 (0.3 mg/mL) were incubated in a reaction mixture containing 1 milliunit/mL casein kinase II, 20 mM MES buffer (pH 7.2), 130 mM KCl, 0.4 mM $MgCl_2$, 48 mM DTT, and 100 μM ATP, in a total volume of 1 mL at 25 °C for 30 min. For the cdc2 kinase phosphorylation, the B23 samples were incubated at 25 °C for 45 min in a buffer containing 0.1 μg of cdc2 kinase, 50 mM Tris-HCl (pH 7.4), 0.4 mM $MgCl_2$, 1 mM EGTA, 200 μM ATP, and 1 μg/mL each of aprotinin, leupeptin, and pepstatin. After phosphorylation, the samples were dialyzed in microdialysis flow cells against 20 mM HEPES buffer (pH 7.4). Aliquots (50 μL) from the incubation mixtures supplemented with [γ-^{32}P]-ATP were routinely used to measure the efficiency of the enzyme reactions and to estimate the number of phosphoryl groups incorporated. Termination of phosphorylation in these aliquots was achieved by addition of 10% TCA followed by two more washes in 10% TCA, with subsequent washes with methanol and ether. The radioactivity incorporated into the protein was measured in a liquid scintillation counter. The number of phosphoryl groups incorporated into the protein was calculated from the radioisotope incorporation, the specific activity of the [γ-^{32}P]ATP, and the amount of protein as determined by the Bio-Rad protein assay.

RESULTS

Binding of the SV40 T-Antigen NLS to Proteins B23.1 and B23.2. Previously, it was shown that peptides based on the NLS of the SV40 T-antigen bind protein B23 with relatively high affinity (Goldfarb, 1988). However, neither the binding constants nor the stoichiometries of these interactions have been determined. Furthermore, it is not known whether the two isoforms differ in their affinities for SV40 T-NLS peptides. To answer these questions, equilibrium dialysis experiments were carried out using a synthetic peptide (T-NLS) containing the SV40 T-antigen NLS (Table 1). In addition to the NLS sequence, this peptide contained Tyr and Cys at the C-terminal end for the purpose of labeling either by iodination of the tyrosine or for attaching a labeling group via the free sulfhydryl group of the carboxyl-terminal cysteine residue. The T-NLS peptide and all other peptides used in this study were labeled with [^{3}H]NEM by the latter route.

Figure 1 shows that labeled T-NLS was able to bind both isoforms of protein B23. The binding was dependent on

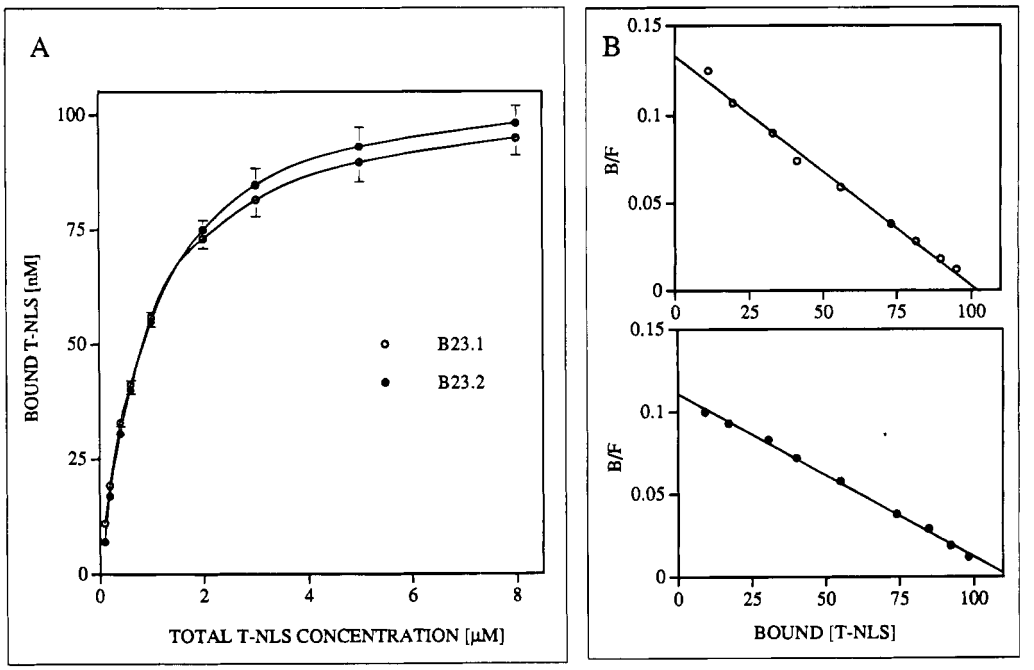

FIGURE 1: Binding of peptide containing the SV40 T-antigen nuclear localization signal (T-NLS) to recombinant protein B23 isoforms. Proteins B23.1 or B23.2 (100 nM) were subjected to equilibrium dialysis against various concentrations of the ^{3}H-labeled T-NLS peptide (Table 1) as described under Experimental Procedures. (A) Saturation curve after titration of B23.1 or B23.2 with the T-NLS. The data are shown as the mean ± SE from six determinations. (B) Scatchard plots of the interaction of ^{3}H-labeled T-NLS peptide with protein B23 isoforms. The bound peptide concentrations are micromolar. The curves in panel B are derived from the data in panel A.

Table 2: Binding Affinities of NLS-Containing Peptides for Protein B23 Isoforms

	K_D (μM)[a]					
peptide	B23.1	B23.2	B23.1-P[c] (CKII)	B23.2-P (CKII)	B23.1-P (cdc2)	B23.2-P (cdc2)
T-NLS	0.824 ± 0.016	0.973 ± 0.023	0.407 ± 0.005	0.381 ± 0.007	0.831 ± 0.014	0.840 ± 0.025
rNLS[b]	1.065 ± 0.788	1.028 ± 0.604	1.248 ± 0.777	0.777 ± 0.616	1.249 ± 0.674	1.154 ± 0.529
Rev37−47	0.082 ± 0.002	0.096 ± 0.003	0.050 ± 0.004	0.067 ± 0.005	0.083 ± 0.003	0.085 ± 0.003
Rev40−47	0.272 ± 0.027	0.366 ± 0.007	0.251 ± 0.021	0.219 ± 0.012	0.338 ± 0.022	0.317 ± 0.012

[a] The dissociation constants ± standard deviations of the interactions of proteins B23.1 or B23.2 with the peptides shown in Table 1 (labeled with [^{3}H]-*N*-ethylmaleimide) were determined by equilibrium dialysis, and the data were fit by nonlinear least-squares analyses using a model that assumes independent noninteracting sites. Standard deviations of the K_Ds were determined from the fits. Each data point was the average of at least six determinations. The standard error for each point was less than 5%. [b] For the reverse sequence NLS, the K_Ds were determined by fitting the binding isotherms to a two-site model. Only the K_Ds for the high-affinity site are shown. The K_Ds for a putative low-affinity site were in a range of 23−82 μM. [c] Proteins B23.1 or B23.2 were phosphorylated either with casein kinase II (CKII) or with a cdc2-type protein kinase (cdc2) as indicated in parentheses below the phosphorylated protein name. Approximately 1.5 and 1 phosphoryl groups per mole of protein were incorporated using CKII or cdc2 kinase, respectively.

the concentration of added peptide and saturable for either protein isoform. The binding curves in Figure 1A suggested that B23.1 and B23.2 have similar affinities for the T-NLS peptide. Scatchard plots were linear (Figure 1B) and yielded dissociation constants for the interactions between the T-NLS and B23.1 or B23.2 of approximately 1 μM. Nonlinear least-squares fits of the binding isotherms to a single-site model yielded K_Ds for the binding of the T-NLS to B23.1 and B23.2 of 0.824 ± 0.016 and 0.973 ± 0.023, respectively (Table 2). Thus, the T-NLS binding affinities were similar for both isoforms. The stoichiometry of binding for both protein B23 isoforms, calculated from the Scatchard plots and the fits, resulted in a 1:1 ratio of moles of peptide bound per mole of protein.

Since the sequence of the seven minimum residues of the NLS of the SV40 T-antigen is critical for nuclear import, it was important to determine whether alteration of the sequence would change the affinity or the stoichiometry of binding to protein B23. To answer this question, a peptide with a sequence which was the reverse of the T-NLS was synthesized (rNLS, Table 1). This peptide was also designed to determine the importance of the arrangement of amino acids relative to the total charge carried by the peptide. Eqilibrium dialyses was performed as in the above experiments using the rNLS. Figure 2 shows that the binding curve of the rNLS interaction with B23.1 had different characteristics than the curves obtained using the T-NLS. Furthermore, the Scatchard plot was nonlinear, suggesting multiple binding sites with different affinities. The binding isotherms were best fit assuming a model with two classes of noninteracting sites and yielded K_Ds of approximately 1 μM for the first class of sites or similar to the value obtained for the T-NLS (Table 2). However, the second class of site had 20−80-fold lower affinity than the T-NLS and an apparent stoichiometry greater than 2:1 (data not shown).

Although the T-NLS and the rNLS had similar affinities for the first site bound, the shapes of the binding isotherms suggested that the binding characteristics were very different

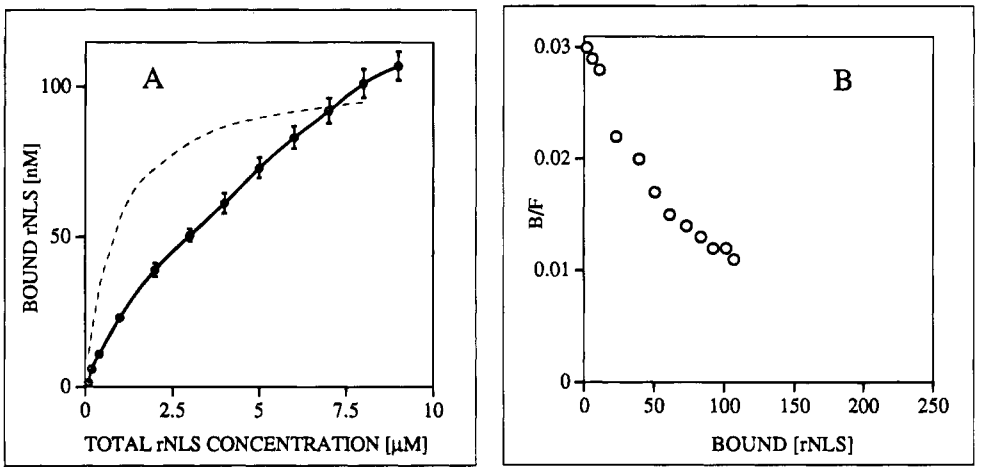

FIGURE 2: Binding of peptide containing the reverse sequence of the SV40 T-antigen nuclear localization signal (rNLS) to protein B23.1. Equilibrium dialysis with the rNLS peptide was carried out as in Figure 1. (A) Saturation curve after titration of recombinant B23.1 with various concentrations of the rNLS peptide (solid line). The data are shown as the mean ± SE from eight determinations. The isotherm for the binding of the T-NLS to B23.1 as in figure 1A is included for comparison (dashed line). (B) Scatchard plot of the interaction of ^{3}H-labeled rNLS peptide with B23.1. The bound peptide concentrations are micromolar. The curve in panel B is derived from the data in panel A.

for the two peptides. Therefore, competition experiments were done to determine whether the two peptides were binding at the same site. Increasing concentrations of unlabeled T-NLS were able to displace labeled T-NLS with 50% displacement achieved at equimolar concentrations of competitor, as expected (data not shown). However, unlabeled rNLS added to the binding mixture had no effect, indicating that it was binding to a different site (or sites) than the T-NLS. These results showed that the sequence of the peptide is important in determining the specificity of binding. The fact that the T-NLS and the rNLS have the same net charge indicates that the specific interaction involves more than simple electrostatic forces.

Interaction of Other NLS Peptides with Protein B23 Isoforms. To determine whether protein B23 has selectivity in binding NLS sequences, three additional synthetic peptides were used for binding assays (Table 1). Two of these were based on the NLS of the HIV-Rev protein (Rev37−47and Rev40−46). The former contains the sequence necessary for nucleolar localization of the Rev protein, and the latter contains only that segment of the Rev protein required for location in the nucleus (Cochrane et al., 1990). The third peptide (npNLS) was derived from the sequence of the bipartite NLS of nucleoplasmin (Robbins et al., 1990). The Rev37−47 peptide had an affinity for either B23 isoform approximately 10-fold higher than the T-NLS (Table 2). However, the interactions of the shorter form of the Rev NLS (Rev40−46) with the protein B23 isoforms had dissociation constants between those seen for the T-NLS and Rev37−47 peptides. The higher affinity of the longer form of the Rev NLS for protein B23 may account for this sequence causing the Rev protein to locate in the nucleolus rather than in the nucleoplasm.

In contrast to the relatively high affinities of the T-NLS and the two Rev-derived peptides for B23, there was no detectable binding of the npNLS to either B23 isoform. This suggests that protein B23 selectively interacts only with the class of NLS sequences based on the SV40 T-antigen and not with bipartite NLS sequences.

Since the longer Rev peptide had a much higher affinity for protein B23 than the T-NLS, it was considered important to determine whether both peptides bind to the same site on the protein. To answer this, we devised a competition experiment in which labeled T-NLS was incubated with B23 in the equilibrium dialysis cell and then unlabeled Rev37−47 was added at various concentrations. When the samples reached equilibrium, the Rev37−47 peptide was capable of displacing the T-NLS in a concentration-dependent manner (data not shown) suggesting that both peptides bind to a single binding site on protein B23.

Phosphorylation of Protein B23 by Casein Kinase II Enhances NLS Binding Affinity. The major site of phosphorylation in protein B23 by casein kinase II (serine 125) is contained in the first highly acidic segment of the protein (Chan et al., 1990). Another potential site is located at threonine 185, which is present in the second highly acidic segment. Since this region of the molecule is a proposed site of interaction with the NLS peptides, we examined the effects of phosphorylation on the affinity of the NLS peptides for the protein B23 isoforms. After phosphorylating either B23.1 or B23.2 with casein kinase II, it was determined that 1.4−1.5 phosphoryl groups were incorporated per protein molecule. This suggested that the major CKII site was completely phosphorylated and the second site was partially phosphorylated, although no attempt was made to determine the locations of the sites. The two phosphorylated forms of protein B23 were subjected to equilibrium dialysis against the various NLS-containing peptides. Table 2 shows that the dissociation constants for the interaction of either form of B23 with the T-NLS or the Rev37−47 NLS decreased approximately by a factor of 2 when the proteins were phosphorylated by casein kinase II. In contrast, there was no significant change in the K_D for the binding of the rNLS to the B23 isoforms, further supporting the idea that the reverse sequence NLS binds nonspecifically to sites outside the normal binding site.

Protein B23 is also known to be phosphorylated in the C-terminal region by the cdc2-type protein kinase. To determine whether the effect seen with CKII was specific for NLS binding, the protein B23 isoforms were phosphorylated with a cdc2 protein kinase. Approximately one phosphoryl group per molecule of B23 was incorporated

using the latter enzyme. When proteins B23.1 and B23.2 were phosphorylated as such, there was no significant change in affinity for any of the NLS peptides tested (Table 2). Thus, the increased affinity of protein B23 for NLS peptides is induced by phosphorylation of residues in the acidic segments of the center of the molecule but not by phosphorylation of cdc2-type sites in the C-terminal end.

DISCUSSION

We have used equilibrium dialysis to determine the dissociation constants for the interaction of two forms of protein B23 with ^{3}H-labeled NLS-containing peptides. We used peptides in these studies because binding assays of this kind cannot be done easily with whole proteins. Peptides based on NLS sequences mimic the NLS segments present in whole proteins; i.e., linking NLS peptides to nonnuclear proteins causes them to be imported into the nucleus (Michaud & Goldfarb, 1993; Boulikas, 1994). Furthermore, a reverse sequence NLS is not capable of competing with this import (Michaud and Goldfarb, 1993), indicating there is specificity in this interaction. Therefore, the use of NLS-containing peptides seems to be a reasonable model for studying the interactions of NLS sequences with acceptor proteins.

In these studies, both recombinant forms of protein B23 bound NLS-containing peptides with K_Ds in the range of 0.1−1 μM. This indicates that the unique C-terminal end of B23.1 does not play an important role in NLS binding and that the NLS binding site is in the segment common to both proteins. The C-terminal extension, found only on B23.1, is important for nucleic acid binding (Wang et al., 1994) and appears to direct B23.1 to the nucleolus (Wang et al., 1993). Protein B23.1, which is expressed at a much higher level than B23.2, would be expected to attract NLS-containing proteins to the nucleolus, where it is predominantly located (Wang et al., 1993). The shorter form, B23.2, might direct NLS-containing proteins to locations other than the nucleolus.

With a p*I* near 5 (Lischwe et al., 1979), protein B23 carries a strong net negative charge at neutral pH. Are the positively charged NLS peptides simply binding to the negatively charged protein by electrostatic forces without any specificity? Studies using a peptide containing the reverse sequence of the SV40-T NLS suggest that factors in addition to electrostatic forces contribute to the affinity and specificity of NLS binding. The reverse sequence peptide bound to protein B23 at multiple sites and exhibited a nonlinear Scatchard plot. In contrast, the Scatchard plot using the correct-sequence peptide was linear with a stoichiometry of 1:1. Futhermore, competition experiments indicated that the reverse sequence peptide bound to different sites on the protein than the correct sequence peptide. Since the two peptides have the same charge, the arrangement of the amino acids must determine the specificity of binding. A peptide containing the nucleoplasmin NLS also did not detectably bind to protein B23. The npNLS actually carries a greater positive charge than the SV40 T-NLS (eight vs five lysine and arginine residues). Thus, in the two cases above, the contribution of the sequence of amino acids appears to be greater than the net charge in determining the affinity of NLS binding.

The finding that there was no detectable binding of the nucleoplasmin NLS by protein B23 is striking. Although there appear to be two classes of NLS sequences, those of the SV40 T-antigen type and those of the bipartite nucleoplasmin type, both classes seem to be imported into the nucleus by a single pathway (Michaud & Goldfarb, 1993). However, this does not mean that the two classes of NLS have the same nuclear acceptors. It is tempting to speculate that protein B23 is able to distinguish between the two classes and direct only the SV40 T-class to the nucleolus.

Protein B23 has been shown to bind the HIV-1 Rev protein and has been proposed to be its nucleolar acceptor (Fankhauser et al., 1991). This is consistent with the observation that the Rev protein is predominantly located in the nucleolus of HIV-1-infected cells (Kubota et al., 1989; Cochrane et al., 1990). Interestingly, a peptide containing the nucleolar localization signal (Rev37−47) of the Rev protein had an affinity for protein B23 approximately 10-fold greater than that of the SV40 T-NLS. The Rev peptide also was able to compete with the SV40 T-NLS, indicating that both peptides bind at the same site on protein B23. The high affinity of this segment of the Rev protein may be an explanation for the localization of the Rev protein in the nucleolus. Deletion of a portion of this region of the Rev protein causes it to be located in the nucleoplasm and largely excluded from the nucleolus (Cochrane et al., 1990). The affinity of a peptide with a truncated nucleolar localization sequence (Rev40−46) binds to protein B23 with an affinity between that of Rev37−47 and the SV40 T-NLS. This lowered affinity could account for the shift of the Rev protein from the nucleolus to the nucleoplasm when the nucleolar localization signal is shortened.

The highly acidic regions of protein B23 are the most likely candidates for binding of NLS sequences (Goldfarb, 1988; Adachi et al., 1993; Xue & Mélèse, 1994). Phosphorylation by casein kinase II sites in these segments of the molecule (Chan et al., 1986a) could modulate the affinity of NLS binding, possibly by strengthening the electrostatic interactions. Indeed, phosphorylation of 1−2 sites by CKII enhanced the binding of the NLS peptides approximately 2-fold. Proteins entering the nucleus appear to be retained on the basis of their affinity for a nuclear acceptor (Schmidt-Zachmann et al., 1993). This change in affinity could shift the equilibrium sufficiently to allow retention of NLS-containing proteins in the nucleolus; e.g., ribosomal proteins. Casein kinase II appears to be especially abundant in the nucleolus (Olson et al., 1978; Belenguer et al., 1989): its proximity to protein B23 could cause the protein to be highly phosphorylated in the nucleolus. Furthermore, CKII activity is greatly enhanced in proliferating cells and tissues (Issinger, 1993), presumably in response to the need for production of ribosomes. Because of its nucleolar location, phosphorylated protein B23.1 could be a major participant in transiently holding these proteins at the sites of ribosome assembly.

Although protein B23 shuttles in and out of the nucleus (Borer et al., 1989), it has never been shown to carry proteins during shuttling. Thus, there is no direct evidence for this protein participating directly in nuclear import of other proteins. However, it could serve as a nuclear or nucleolar acceptor for NLS-containing proteins, without direct involvement in import. Development of new experimental approaches will be required to define the precise role of this protein in transport of nuclear proteins.

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