High-Throughput Protein Sequencing

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High-Throughput Protein Sequencing

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We have designed and implemented an autosampler that provides additional sample capacity on a commercial protein sequencer. The autosampler attaches to a standard ABI Procise sequencer, enabling a single-sample cartridge to hold up to six separate samples. The autosampler is used in combination with faster Edman cycles and a rapid 12-min PTH separation to significantly increase the speed of automated protein sequencing. We also describe Edman chemistry modifications that improve coupling efficiency and prevent internal cleavage that can occur when samples are sequenced in the standard glass cartridges.

Edman degradation is a frequently utilized methodology for the characterization of recombinant proteins. Automated protein sequencing can provide direct confirmation of the N-terminal sequence as well as provide a quantitative measure of ragged N-termini, the presence of an unprocessed signal sequence, and the sequences of internal cleavage sites. These measurements are often difficult to obtain with other techniques such as peptide mass fingerprinting.[1−5] We have previously reported a 20-min Edman chemistry cycle that utilizes a 12-min phenylthiohydantoin (PTH) separation.[6] In this paper, we describe a protein sequencer autosampler that increases the number of samples from four to nine on the Applied Biosystems Procise protein sequencer using the rapid Edman cycle. This autosampler can be constructed from readily available components with some minor machining. The small compact cartridge assembly replaces one of the standard glass cartridge holders.

EXPERIMENTAL SECTION

Materials. HPLC-grade acetonitrile and methanol were from Burdick and Jackson (Muskegon, MI). Dithiothreitol was obtained from Diagnostic Chemicals (Oxford, CT). *N*-Isopropyl iodoacetamide was from Molecular Probes (Eugene, OR). Thioglycolic acid (mercaptoacetic acid), 3-(cyclohexylamino)-1-propanesulfonic acid and electrophoresis purity 2-mercaptoethanol were purchased from BioRad (Hercules, CA). Glacial acetic acid and sodium phosphate were from Fluka Chemical Corp. (Milwaukee, WI). All water used was from an Ultrapure Milli-Q system by Millipore (Milford, MA).

Solvents A (5% tetrahydrofuran), B (12% 2-propanol in acetonitrile), PreMix Buffer Concentrate, and amino acid PTH Standard were from Applied Biosystems (Foster City, CA). PTH amino acids were separated using 2.0 × 100 mm columns LS-1021-C183 containing 3-μm Haisil resin supplied by Higgens Analytical, Inc. (Mountain View, CA). Guard columns (1 mm, 10-02-00014) containing silica were obtained from Optimize Technologies, Inc. (Oregon City, OR).

Alkylation and Electroblotting. Proteins were reduced in 20 μL of BioRad Laemmli sample buffer adjusted to pH 8.3 containing 10 mM DTT at 85 °C for 5 min. Alkylation was performed by the addition of 2 μL of 200 mM (0.08 mg) *N*-isopropyliodoacetamide in methanol followed by incubation in the dark at 25 °C for 20 min.[8] Proteins were separated on BioRad precast gels and electroblotted on Applied Biosystems Problott membranes in a BioRad Trans-Blot transfer cell using 10 mM 3-(cyclohexylamino)-1-propanesulfonic acid, pH 11.0, 10 mM thioglycolic acid, 10% methanol as the transfer buffer for 1 h at 250-mA constant current.[9] The PVDF membrane was stained with 0.1% Coomassie Blue R-250 in 50% methanol for 0.5 min and destained with 10% acetic acid in 50% methanol for 2−3 min. The membrane was thoroughly washed with water and allowed to dry before storage at −20 °C.

Multiple Cartridge Assembly. The Applied Biosystem Procise 494A sequencer was equipped with six Teflon tube horizontal reaction cartridges ($^1/_{16}$-in. i.d., $^1/_8$-in. o.d, 1 in. long) in a rectangular aluminum block $^5/_8$ in. wide and 1.25 in. long that was machined to accommodate the Teflon tubing. The aluminum blocks were heated by 0.5 × 1.25 in. Minco (Minneapolis, MN) thermofoil heaters (model HK5575R20.9L12E). The heaters were controlled by an Omega temperature controller (CN7613-8PV). Optical sensors (part 4798) were obtained from Applied Biosystems. Cartridge selection was controlled by a 14-port stainless steel model EMT-CST6-UWTF automated Valco valve (Houston, TX) equipped with a Teflon rotor (SSACS-T6UWTF) and a multiposition actuator control module (model EMTCA), which was controlled by an external relay in the protein sequencer. Gas flow measurements were obtained using an ADM-1000 flowmeter (Alltech).

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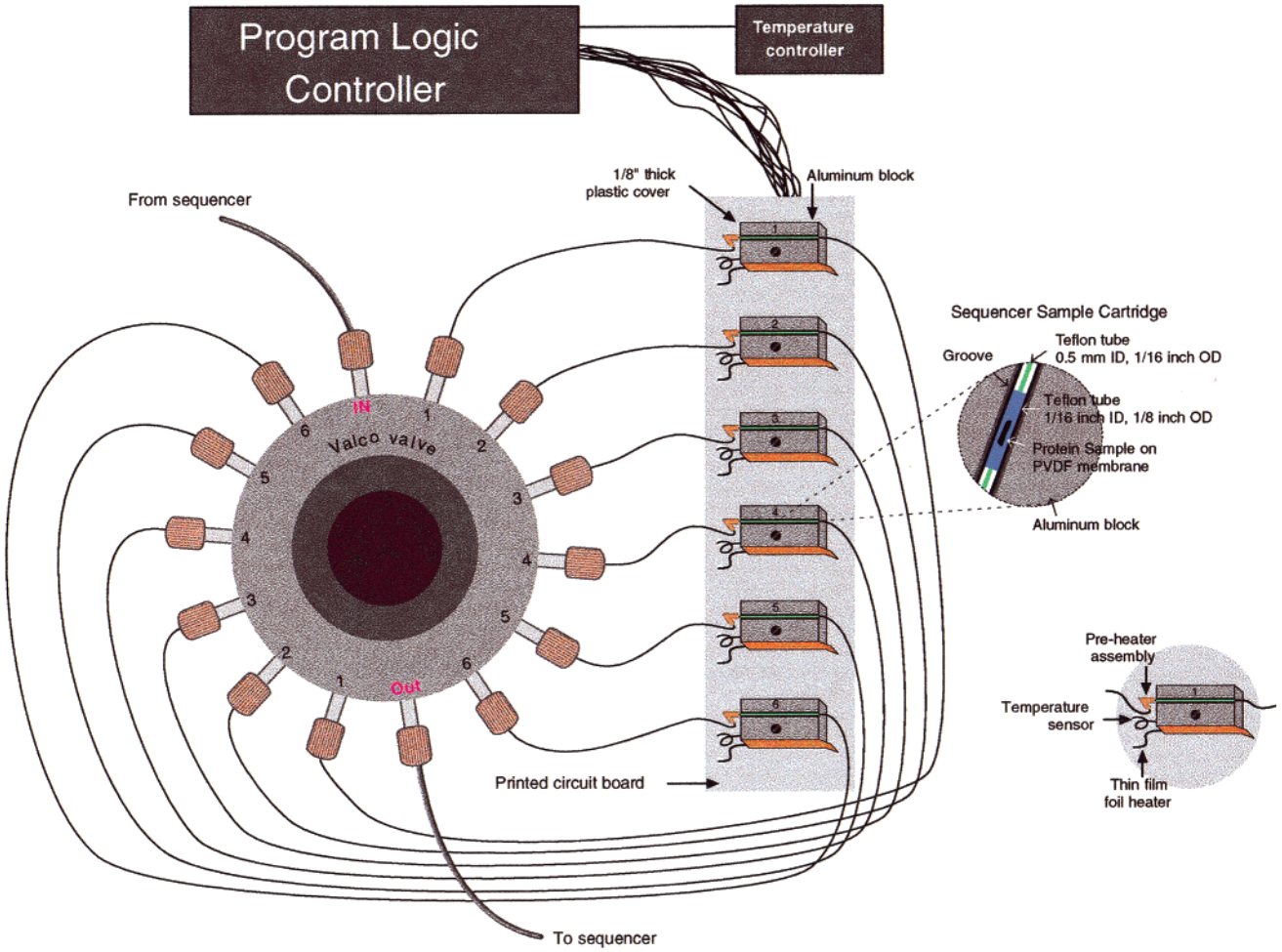

Figure 1. Schematic diagram showing the multiple-sample Teflon horizontal flow reaction cartridge system.

Program Logic Controller (PLC). A PLC model DL205 from Automation Direct (Cumming, GA) was used to select the temperature and optical sensors, heater, and Valco valve position for each Teflon reaction cartridge. The PLC consisted of a Windows CE CPU (H2-WPLC1), 8-point 12−24-V input module (D2-80ND3), three 12-point 1.5A relay modules (D2-12TR), an Ethernet communication module (H2-ECOM), and a 6-slot base (D2-06B). The PLC was programmed using Entivity's Think and Do Live software from Automation Direct.

Automated Edman Degradation. Automated protein sequencing was performed on Applied Biosystems Procise 494A protein sequencers. The Procise sequencers were equipped with 6-mm-diameter microcartridges and an on-line PTH analyzer. The coupling buffer (R2) was *N*-methylpiperidine in 1-propanol and water (25:60:15) supplied by Applied Biosystems or distilled in-house. Twenty-minute Edman cycles were used as described[6] with the following modification. A high-pressure (3.0 psi) delivery of R2 coupling buffer was delivered for 20 s prior to all R1 deliveries. Acetone was routinely added to solvent A to balance the baseline. Peaks were integrated with Chromperfect from Justice Innovation software. Sequence interpretation was performed on a DEC Alpha.[10]

SEQSORT. The SEQSORT algorithm was used to sort sequence mixtures.[6,11] The algorithm finds patterns specified as regular-expression syntax. Sequence mixtures can be sorted using a known sequence or by comparing the sequence mixture with all proteins in a protein sequence database.

RESULTS AND DISCUSSION

Reaction Cartridge Assembly. The protein samples were electroblotted onto PVDF membranes prior to sequencing. The bands of interest were excised from the membrane and placed on the autosampler in reaction cartridges composed of disposable Teflon tubing ($^1/_{16}$-in. i.d., $^1/_8$-in. o.d., ∼1 in. long). The disposable Teflon tubing reaction cartridges allowed for a significantly cleaner background when compared to the reusable glass cartridge blocks that are used on the ABI sequencers.[6−7] The lower background reduced the ambiguity of identifying the amino acids in the first few cycles, which is often problematic.

Figure 1 shows a schematic diagram of the multiple cartridge assembly. The sample-containing $^1/_8$-in.-o.d. Teflon tubing was connected to two 0.5-mm-i.d., $^1/_{16}$-in.-o.d. lengths of Teflon tubing originating from and returning to a Valco valve. The connection is a simple press fit. This connection must be leak tight and can

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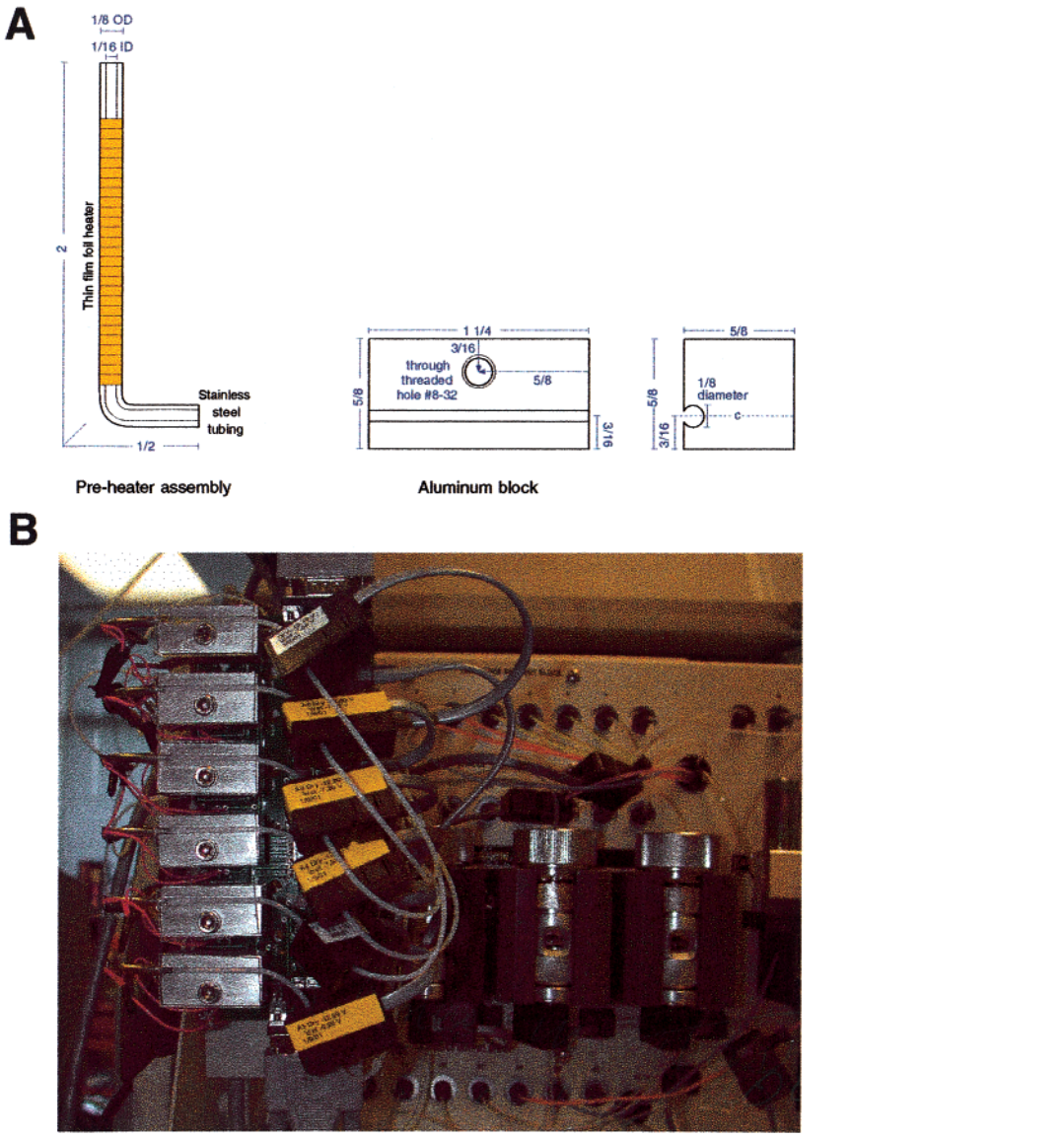

Figure 2. (A) Diagram of the aluminum reaction cartridge holder and the preheater assembly. (B) Photograph of the multiple cartridge assembly mounted on the Procise sequencer.

be verified by using the ABI cartridge leak test procedure after selecting the proper Teflon reaction cartridge. A Valco valve controller allows manual control of cartridge selection.

The Teflon reaction cartridge was fitted into a recess of a machined aluminum block, which serves as a heat sink. Each block contains an individual thin-film foil heater and thermocouple. The small size of this block did not provide sufficient thermal mass to prevent cooling from the gas-phase delivery of the coupling buffer (R2). A short piece of 0.25-in.-i.d. stainless steel tubing (preheater) equipped with a separate thin-foil heater connected in series with the reaction block heater solved this problem. (Figure 2A). This metal tubing preheats all reagents that enter the reaction cartridge. The preheater fits a short distance ($^1/_4$ in.) into the reaction cartridge aluminum block. The six cartridges were mounted on a printed circuit board, which served to simplify the wiring as well as to provide a compact assembly that could fit into the space occupied by a standard glass block cartridge. The number of cartridges that were mounted to the circuit board was based on the available space occupied by an existing glass

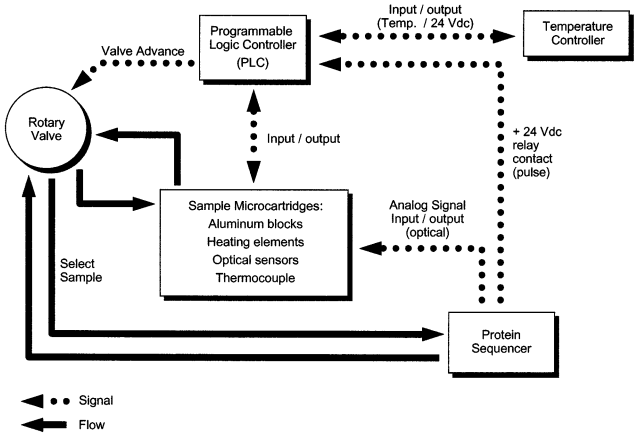

Figure 3. Block diagram showing the interconnections between the sequencer, Valco valve, and PLC.

cartridge block. The multicartridge assembly was inserted into an existing cartridge slot on the Procise HT sequencer, allowing for six samples to be analyzed in the place of one (Figure 2B).

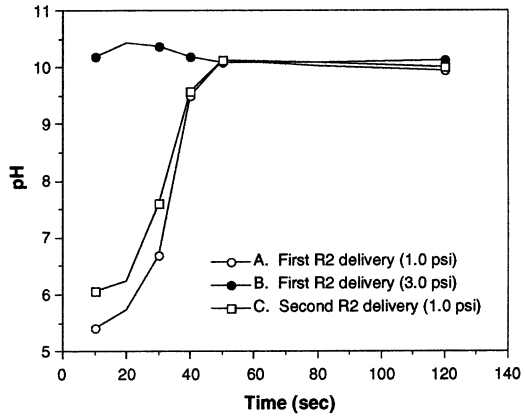

Figure 4. pH measurement during the PITC coupling reaction. (A) The first R2 delivery at 1.0 psi; (B) first R2 delivery using a 20-s 3 psi R2 delivery followed by a delivery at 1.0 psi; (C) second R2 delivery at 1.0 psi.

Table 1. Effect of Sample Placement on Sequencer-Derived Cleavage for Human Small Inducible Cytokine A7 Precursor IgG Fusion

	N-terminal sequence QPVGINTSTTC	Thr cleavage TCPPAPELL
Cart A	+	–
Cart C (after Cart A)	+	–
Cart B (after Cart A)	+	29%
Cart C (after Cart A, B)	+	25%
Cart C (after Cart A, B) + ST[a]	+	–
Cart D (after Cart B, C) + ST[a]	+	–

[a] ST is the startup procedure that is used when all samples have been loaded on the sequencer. This procedure delivers a 3.0 psi R2 delivery for 30 s to all cartridges prior to sequencing. All cartridges above were run for 11 cycles.

Table 2. Startup Procedure: Del R2 Multi A1–6

step	fxn no.	function name	time/ temp	El. time
1	258	begin	0	:00
2	303	select regulator	2	:00
3	304	save regulator set point	0	:00
4	305	set reg set point (10th psi)	30	:00
5	123	select cartridge a	0	:00
6	11	del R2g, Cart (top)	30	:30
7	255	490A Relay 2 on	1	:31
8	257	wait	1	:32
9	254	490A relay 2 off	1	:33
10	11	del R2g, Cart (top)	30	1:03
11	255	490A relay 2 on	1	1:04
12	257	wait	1	1:05
13	254	490A relay 2 off	1	1:06
14	11	del R2g, Cart (top)	30	1:36
15	255	490A relay 2 on	1	1:37
16	257	wait	1	1:38
17	254	490A relay 2 off	1	1:39
18	11	del R2g, Cart (top)	30	2:09
19	255	490A relay 2 on	1	2:10
20	257	wait	1	2:11
21	254	490A relay 2 off	1	2:12
22	11	del R2g, Cart (top)	30	2:42
23	255	490A relay 2 on	1	2:43
24	257	wait	1	2:44
25	254	490A relay 2 off	1	2:45
26	11	del R2g, Cart (top)	30	3:15
27	255	490A relay 2 on	1	3:16
28	257	wait	1	3:17
29	254	490A relay 2 off	1	3:18
30	124	select cartridge B	0	3:18
21	11	del R2g, Cart (top)	30	3:48
32	124	select cartridge C	0	3:48
33	11	del R2g, Cart (top)	30	4:18
34	124	select cartridge D	0	4:18
35	11	del R2g, Cart (top)	30	4:48
36	309	restore reg set point	0	4:48
37	147	end cartridge select	0	4:48
38	259	end	0	4:48

Additional capacity could be added by replacing the remaining glass blocks with more multicartridge assemblies or more Teflon cartridges could be added to a multicartridge unit by using a valve containing additional ports. Each of six blocks may be selectively heated when sequencing on that sample begins. The Valco valve controls the flow of sequencing reagents into the appropriate cartridge block.

A low-cost PLC was connected through an external relay to the protein sequencer and serves to control the autosampler. The PLC selects the cartridge, optical sensor, heater, and thermocouple for each cartridge. Optical sensors are connected to the Teflon tubing exiting the Teflon reaction cartridges and contain an infrared diode and photocell, which are used to precisely control the delivery of solvents and reagents to the Teflon cartridges. The sensors are used to detect the presence of liquid in the Teflon tubing delivery lines and are connected to a microprocessor in the protein sequencer, which stops the delivery of reagent or solvent when a threshold voltage value is reached. The optical sensors used for the Teflon cartridges are the same optical sensors used on the Procise sequencer. The output of the optical sensors from the printed circuit board is plugged into the port for the optical sensor for the previously removed glass cartridge block. The PLC selects the optical sensor corresponding to the Teflon cartridge in use. In this way, a single optical sensor port on the Procise sequencer is multiplexed to serve six cartridges.

A block diagram showing the interconnections between the protein sequencer, Valco valve, and PLC is shown in Figure 3. The PLC input module was connected to a contact closure on the Procise sequencer, and a contact closure event was added to the end of each sequence cycle, providing a method of cycle counting for the PLC device. The PLC program allows the cartridges to be sequenced in any order. Each cartridge can be programmed individually with the number of cycles desired. Cartridges are only heated when sequencing on a cartridge is in progress. The PLC was programmed with the number of cycles for each cartridge. After reaching the preprogrammed cycle number for cartridge one, the PLC automatically advances to the Valco position for cartridge two and switches from heater and thermocouple one to the thermocouple and sensor contacts for cartridge two. After sequencing has been completed on all programmed cartridges, the last cartridge heater is turned off and the Valco valve is set to cartridge one. The PLC is connected to a Ethernet network and can be controlled by any PC computer on the network that is running Think & Do Live software.

Edman Chemistry Modifications. Figure 4 shows the pH drop that occurs after phenyl isothiocyanate (PITC) is delivered to the reaction cartridge. This phenomenon was observed on three

Table 3. Cartridge and Flask Cycle Listing for the Teflon Cartridges

	cycle: multifast blot				flask cycle: flask STD				flask cycle: flask fast residue			
step	fxn no.	function name	time/ temp	E1 time	fxn no.	function name	time/ temp	E1 time	fxn no.	function name	time/ temp	E1 time
1	258	begin	0	0:00	258	begin	0	:00	258	begin	0	:00
2	136	flush Cart solvent block	10	:010	234	set as standard cycle	0	:00	235	set as residue cycle	0	:00
3	137	flush input block	10	0:20	181	del X2, flask	25	:25	218	flush large loop (flask)	15	:15
4	135	flush Cart reagent block	10	0:30	213	dry flask	5	:30	173	load S4, flask (lg loop)	12	:27
5	303	select regulator	2	0:30	215	empty flask	20	:50	213	dry flask	10	:37
6	304	save regulator set point	0	0:30	157	del R4, waste	10	1:00	218	flush large loop (flask)	15	:52
7	305	set Reg set point (10th psi)	30	0:30	218	flush large loop (flask)	15	1:15	228	ready to receive	1	:53
8	11	del R2g, Cart (top)	30	1:00	163	load R5, flask (lg loop)	15	1:30	213	dry flask	5	:58
9	140	flush large loop (Cart)	10	1:10	213	dry flask	60	2:30	236	preconversion dry	25	1:23
10	6	load R1, cart (lg loop)	15	1:25	218	flush large loop (flask)	15	2:45	218	flush large loop (flask)	15	1:38
11	131	dry Cart (top)	20	1:45	153	load R4, flask (lg loop)	20	3:05	153	load R4, flask (lg loop)	20	1:58
12	140	flush large loop (Cart)	10	1:55	213	dry flask	10	3:15	213	dry flask	10	2:08
13	135	flush Cart reagent block	10	2:05	218	flush large loop (flask)	15	3:30	218	flush large loop (flask)	15	2:23
14	11	del R2g, Cart (top)	20	2:25	173	load S4, flask (lg loop)	12	3:42	173	load S4, flask (lg loop)	12	2:35
15	309	restore Reg set point	0	2:25	218	flush large loop (flask)	15	3:57	218	flush large loop (flask)	15	2:50
16	11	del R2g, Cart (top)	100	4:05	212	bubble flask	2	3:59	212	bubble flask	2	2:52
17	6	load R1, Cart (lg loop)	15	4:20	257	wait	135	6:14	257	wait	135	5:07
18	131	dry Cart (top)	20	4:40	212	bubble flask	5	6:19	212	bubble flask	5	5:12
19	135	flush Cart reagent block	10	4:50	257	wait	105	8:04	257	wait	105	6:57
20	303	select regulator	2	4:50	212	bubble flask	5	8:09	212	bubble flask	5	7:02
21	304	save regulator set point	0	4:50	227	prepare pump	0	8:09	237	prepare pump	1	7:03
22	305	set Reg set point (10th psi)	30	4:50	237	postconversion dry	130	10:19	227	postconversion dry	130	9:13
23	11	del R2g, Cart (top)	20	5:10	213	load position	1	10:20	213	load position	1	9:14
24	309	restore Reg set point	0	5:10	213	dry flask	300	15:20	213	dry flask	300	14:14
25	11	del R2g, Cart (top)	100	6:50	217	flush small loop (flask)	10	15:31	217	flush small loop (flask)	10	14:24
26	131	dry Cart (top)	60	7:50	172	load S4, flask (sm loop)	8	15:38	172	load S4, flask (sm loop)	8	14:32
27	135	flush Cart reagent block	10	8:00	213	dry flask	10	15:48	213	dry flask	10	14:42
28	403	Cart reagent block wash S1	5	8:05	217	flush small loop (flask)	10	15:58	217	flush small loop (flask)	10	14:52
29	135	flush Cart reagent block	10	8:15	218	flush large loop (flask)	15	16:13	218	flush large loop (flask)	15	15:07
30	137	flush input block	10	8:25	173	load S4, flask (lg loop)	12	16:25	173	load S4, flask (lg loop)	12	15:19
31	402	wash (Cart) large loop S1	5	8:30	213	dry flask	10	16:35	213	dry flask	10	15:29
32	140	flush large loop (Cart)	10	8:40	218	flush large loop (flask)	15	16:50	218	flush large loop (flask)	15	15:44
33	53	del S2, Cart (sensor)	20	9:00	221	flush injector	30	17:20	221	flush injector	30	16:14
34	148	cartridge wait	5	9:05	257	wait	2	17:22	257	wait	2	16:16
35	51	del S2, Cart (top)	5	9:10	224	flush injector (low Pres)	20	17:42	224	flush injector (low Pres)	20	16:36
36	148	cartridge wait	5	9:15	213	dry flask	5	17:47	213	dry flask	5	16:41
37	61	del S3, Cart (top)	5	9:20	238	concentrate sample	2	17:49	238	concentrate sample	2	16:43
38	148	cartridge wait	5	9:25	252	490A relay 1 on	1	17:50	252	490A relay 1 on	1	16:44
39	61	del S3, Cart (top)	5	9:30	257	wait	2	17:52	257	wait	2	16:46
40	148	cartridge wait	5	9:35	225	load injector	40	18:32	225	load injector	40	17:26
41	131	dry Cart (top)	60	10:35	223	inject position	1	18:33	223	inject position	1	17:27
42	135	flush Cart reagent block	10	10:45	232	start gradient	0	18:33	232	start gradient	0	17:27
43	137	flush input block	10	10:55	257	wait	2	18:35	257	wait	2	17:29
44	139	flush small loop (Cart)	10	11:05	251	490A relay 1 off	1	18:36	251	490A relay 1 off	1	17:30
45	25	load R3, Cart (sm loop)	30	11:35	181	del X2, flask	25	19:01	181	del X2, flask	25	17:55
46	30	transfer R3, Cart (gas)	5	11:40	213	dry flask	5	19:06	213	dry flask	5	18:00
47	139	flush small loop (Cart)	10	11:50	401	flush flask/inject (high Pres)	40	19:46	215	empty flask	10	18:10
48	404	wash Cart solvent block S1	5	11:55	215	empty flask	10	19:56	401	flush flask/inject (high Pres)	40	18:50
49	136	flush Cart solvent block	10	12:05	221	flush injector	60	20:16	213	dry flask	20	19:10
50	135	flush Cart reagent block	10	12:15	259	end	0	20:16	221	flush injector	60	20:10
51	137	flush input block	10	12:25					259	end	0	20:10

Table 3. (Continued)

	cycle: multifast blot				flask cycle: flask STD				flask cycle: flask fast residue			
step	fxn no.	function name	time/ temp	E1 time	fxn no.	function name	time/ temp	E1 time	fxn no.	function name	time/ temp	E1 time
52	405	wash (Cart) small loop S1	5	12:30								
53	139	flush small loop (Cart)	10	12:40								
54	138	flush output block	10	12:50								
55	257	wait	220	16:30								
56	131	dry Cart (top)	40	17:10								
57	127	ready transfer to flask	0	17:10								
58	141	flush transfer line	5	17:15								
59	63	del S3, Cart (sensor)	20	17:35								
60	148	cartridge wait	5	17:40								
61	121	transfer to flask (gas)	30	18:10								
62	141	flush transfer line	5	18:15								
63	53	del S2, Cart (sensor)	20	18:35								
64	148	cartridge wait	5	18:40								
65	121	transfer to flask (gas)	30	19:10								
66	141	flush transfer line	5	19:15								
67	63	del S3, Cart (sensor)	20	19:35								
68	148	cartridge wait	5	19:40								
69	121	transfer to flask (gas)	30	20:10								
70	141	flush transfer line	10	20:20								
71	128	transfer complete	0	20:20								
72	61	del S3, Cart (top)	10	20:30								
73	148	cartridge wait	5	20:35								
74	131	dry Cart (top)	60	21:35								
75	255	490A relay 2 on	1	21:36								
76	257	wait	2	21:38								
77	254	490A relay 2 off	1	21:39								
78	259	end	0	21:39								

Procise HT and two cLC protein sequencers. The Teflon tubing that delivers PITC also delivers trifluoroacetic acid (TFA), which diffuses into the pores of the Teflon tubing. Washing of the tubing with solvent is ineffective in removing the TFA from the pores of the tubing. The TFA diffuses out of the pores over time and is solubilized by the PITC/heptane reagent. This lowers the pH of the coupling reaction preventing the N-terminal amino acid from reacting with PITC. This problem was corrected by using a high-pressure (3.0 psi) delivery of R2 coupling buffer for 20 s prior to all R1 deliveries. The short duration of high-pressure R2 increases the pH to 10 in 20 s. At the normal pressure of 1.0 psi, 60 s is required to reach pH 10. Since the coupling reaction rate decreases at lower pH, high pH is important to achieve high repetitive yields. We have incorporated the high-pressure R2 delivery on both HT and cLC Procise sequencers. The R2 coupling buffer is a vapor-phase delivery consisting of methylpiperdine and argon gas. It is only necessary to utilize a short duration of high-pressure R2 delivery, followed by the normal delivery of R2 at 1.0 psi. Longer delivery at high pressure can result in the drying of the sample on the PVDF membrane, preventing the reaction of PITC with the N-terminal amino acid.

We have also observed that some proteins will undergo cleavage at the amino side of Thr and Ser residues if placed in a standard glass cartridge next to a heated cartridge. This cleavage occurs from the outgassing of TFA from the pores of the Teflon inlet and outlet reaction cartridge lines and the small amount of heat from an adjacent cartridge that is in use. We have found that immunoglobins are particularly sensitive to this type of cleavage. For example, using an IgG fusion of human small inducible cytokine a7 precursor, cleavage at an internal Thr residue was observed on five different protein sequencers (Table 1), when the sample was placed adjacent to a heated cartridge and then sequenced. However, when the sample is placed two cartridges away from a heated cartridge for several hours prior to sequencing, cleavage does not occur. This problem was completely eliminated by the use of a simple startup procedure (Table 2) that delivers high-pressure R2 to all cartridges to neutralize residual TFA before sequencing begins.

The sequencer method developed here consisted of two standards followed by cartridge cycles (Table 3). The number of cartridge cycles programmed on the Procise sequencer is equal to the sum of all cycles for all multicartridge sample cartridges. Each cartridge cycle ends with a contact closure event that activates a relay. The external relay on the Procise sequencer is connected to the input counter of the PLC. Every time the relay is activated, the PLC advances the cycle counter by 1.

PTH Separation. Using a 3-μm 2 × 100 mm C18 Haisil HL column from Higgens Analytical we were able to separate and resolve all PTH amino acids in the PTH standard from Applied Biosystem in less than 13 min (Figure 5) using the gradient shown in Table 4. The use of a guard column increased column longevity. We have been using Haisil columns for the past two years and have obtained more than 4000 injections on a single column when guard columns were changed every 10 days.

Edman Sequencing. The chromatograms obtained from sequencing human Kunitz-type protease inhibitor 1 on the disposable Teflon cartridge are shown in Figure 5. The sequence found (GPPPAPPGLP) contains a series of adjacent proline residues. Proline residues undergo a slower cleavage reaction in the Edman chemistry than other amino acids, which can result in significant sequence lag. Despite the large number of proline residues that were present in this sequence, the sequence is clearly interpreted without significant carryover. Many other proteins have been sequenced in these disposable Teflon cartridges, demonstrating comparable functionality to the original glass cartridges.

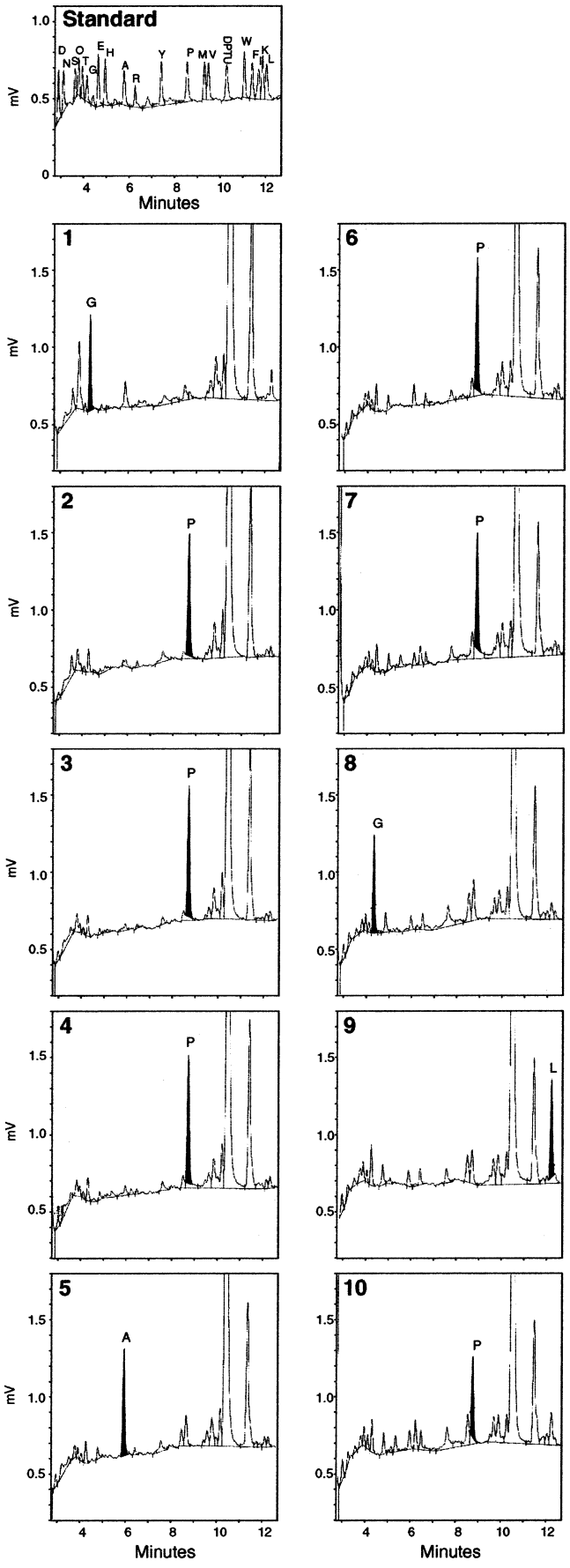

Figure 5. Edman cycles showing the sequence obtained when human Kunitz-type protease inhibitor 1 (Swissprot database accession number SPT1_human) was sequenced on the disposable Teflon cartridge system.

Table 4. HPLC Gradient for the PTH Separation[a]

time	% B	μL/min
0.0	4	275
0.2	4	275
0.3	18	275
12.5	50	275
12.6	90	300
15.6	90	300

[a] Solvent A was 5% tetrahydrofuran containing 25 mL of PreMix Buffer Concentrate, 100 μL of 1 M sodium phosphate, 25 μL pf acetone, and 1 mL of glacial acetic acid. Solvent B was 12% 2-propanol in acetonitrile. Pump parameters: maximum pressure, 2500 psi; minimum pressure, 0; target pressure, 1500 psi; target time, 1 min; data collection time, 20 min.

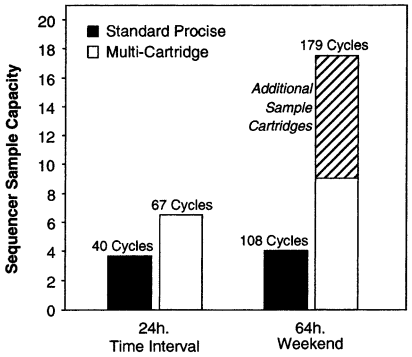

Figure 6. Comparison of protein sequencer sample capacity and fast Edman cycles.

We have eliminated the use of a "begin" cycle, which has been traditionally used to wash a sample before sequencing begins to remove salts and nonvolatile components that might interfere with the Edman chemistry. The salts are removed by washing the sample with organic solvents after acidification with TFA. We found this process is unnecessary with PVDF blots. The staining and destaining of the PVDF membrane removes free glycine and other components of the gel buffers and provides clean samples for sequencing as long as the blots have been handled carefully using gloves. The 33-min "begin" cycle from Applied Biosystems also contains a delivery of PITC. This provides an extra coupling, which we have also found to be unnecessary. Instead of running standards between samples, we run two standards before the samples are run. The elimination of standards between runs and the "begin" cycle with each sample decreases the instrument time necessary to obtain sequence on each sample, thereby increasing sample throughput. Figure 6 summarizes the sample throughput possible using more reaction cartridges and fast Edman cycles. Ten Edman cycles are usually sufficient to identify a protein. Using 10 cycles for each protein sample, six proteins can be analyzed in 24 h using fast Edman cycles. This nearly doubles the throughtput of the standard Applied Biosystems sequencing protocol.

During the weekend, more than 170 cycles could be generated using fast Edman cycles, which would allow analysis of 17 samples. This is more than the nine samples that could be loaded using the six-sample multiple cartridge autosampler and three glass

cartridges on the design presented here and significantly more than the unmodified Procise sequencer with four glass cartridges. Replacing another glass cartridge with an additional multiple cartridge assembly could further increase the capacity of this design. Alternately, the additional cycles that can be generated during a weekend run can be used on samples that require more residues of sequence. For instance, we have utilized longer sequence runs of 15−20 residues to obtain sufficient sequence for cloning of monoclonal antibodies. The combination of additional sample capacity and faster Edman cycles enables protein sequencing to be considered as a high-throughput protein analysis tool.

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SUPPORTING INFORMATION AVAILABLE

The schematic wiring diagram for the PLC and the wiring diagram for the connections between the Valco valve, protein sequencer, and the PLC. This material is available free of charge via the Internet at http://pubs.acs.org.

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