ELSEVIER

Toxicon 43 (2004) 329–335

TOXICON

www.elsevier.com/locate/toxicon

Capillary electrophoresis to characterize ricin and its subunits with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

Dong Hee Na[a], Cheong Kwan Cho[a], Yu Seok Youn[a], Youngju Choi[b], Kang Ro Lee[a], Sun Dong Yoo[a], Kang Choon Lee[a,*]

[a]*Drug Targeting Laboratory, College of Pharmacy, SungKyunKwan University, 300 Chonchon-dong, Jangan-ku, Suwon City 440-746, South Korea*

[b]*Center for Biologics Evaluation, Korea Food and Drug Administration, Seoul, South Korea*

Received 15 September 2003; accepted 6 January 2004

Abstract

Capillary electrophoresis (CE) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) have been employed as highly efficient methods to characterize ricin, its subunits, and the chemically deglycosylated forms. As a CE method, sodium dodecyl sulfate–capillary gel electrophoresis (SDS–CGE) was used because of its merit over the conventional slab gel techniques. SDS–CGE showed higher resolution capability over other analytical tools in the analysis of the ricin mixture as well as in each of its purified forms. The high resolution was considered to be a result of the presence of carbohydrates on ricin subunits, and this property was useful for identifying the native ricin or its A chain from their chemically deglycosylated forms. However, this method exhibited an overestimation of the molecular mass due to the carbohydrate moieties on ricin subunits, and the inaccuracies were observed to be dependent on the carbohydrate content of the subunits. The exact molecular masses were measured by MALDI-TOF MS, and the results were almost consistent with the expected values. This study clearly illustrates the usefulness and necessity of complementary use of two powerful analytical techniques to characterize ricin and its subunits in a various research fields such as poisoning and immunotoxin research.

Keywords: Ricin; Immunotoxin; Capillary electrophoresis; Sodium dodecyl sulfate–capillary gel electrophoresis; Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

1. Introduction

Ricin, which is a plant toxin present in the seeds of the castor bean (*Ricinus communis*), is a heterodimeric glycoprotein composed of a toxic A subunit (RTA) and a galactose-specific lectin B subunit (RTB) via a disulfide bond (Lord et al., 1994). Because of its high toxicity and the potential applications for chemotherapy, the ricin and isolated RTA have been extensively used as immunotoxins by conjugating them to monoclonal antibodies (mAb) (Vitetta et al., 1993; Thrush et al., 1996; Kreitman and Pastan, 1998). To decrease the non-specific binding caused by RTB, the chain A (RTA) alone has been generally coupled with mAbs to prepare immunotoxins (Trown et al., 1991).

Native RTA consists of two forms with identical protein sequences but different apparent molecular weights depending on the carbohydrate contents. Moreover, RTA uptake by macrophage via mannose receptor mediated internalization pathways can be reduced by chemically deglycosylating the toxin and thus reducing its reactivity with the reticuloendothelial system (Blakey et al., 1987). It is reported that the reduced clearance of deglycosylated RTA (dgRTA)

* Corresponding author. Tel.: + 82-31-290-7704; fax: +82-31-290-7724.

E-mail address: kclee@skku.edu (K.C. Lee).

0041-0101/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2004.01.003

at the liver has resulted in improved therapeutic indices of immunotoxins containing dgRTA compared to immunotoxins containing native RTA (Thorpe et al., 1988). In fact, the analysis necessary to characterize the chemically deglycosylated toxins is a substantial challenge. Therefore, the rapid isolation and characterization of ricin or its subunit is very important for the application of ricin or its subunits to therapeutic agents such as immunotoxins.

Although conventional slab gel techniques using silver or Coomassie Blue staining, i.e. SDS–PAGE, offer good separations, they are time-consuming and often do not provide correct results because commercial dyes used for visualizing proteins do not bind to all proteins in a stoichiometric manner (Werner et al., 1993). Moreover, the accuracy of protein identification by SDS–PAGE, ranging from a few percent for a globular protein to which the dye binds properly to about 30% for a heavily glycosylated protein, may not be adequate in many cases (Nguyen et al., 1995).

CE is a rapid micro-analytical technique with high resolution for the identification of biological substances (Karger et al., 1989). From the demonstrated correlation (Guttman and Nolan, 1994; Hunt and Nashabeh, 1999), CE showed advantages such as speed, high resolution, automation, and easy quantifiability demonstrating a great potential for replacing lengthy and tedious slab gel counterparts (Karger et al., 1995; Denton and Tate, 1997). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a recently developed mass spectrometric technique providing an accurate and powerful characterization of peptides and proteins (Kaufmann, 1995). The technique is extremely sensitive, requiring tiny amounts of samples (picomole to subpicomole), and has a detectable mass range in excess of 300 kDa. It also enables precise mass determination ($\pm 0.1\%$) and is relatively insensitive to the presence of buffering agents, salts, and denaturants. These features make it the method of choice for the initial characterization and identification of proteins on the basis of molecular weight (Fenselau, 1997).

Of special interest for the determination of high molecular mass proteins in the biotechnological processes is the complementary use of chromatographic, electrophoretic and mass spectrometric methods such as HPLC, CE and MALDI-TOF MS. The combination of the high resolution and quantifiability of CE and the highly sensitive and precise identification properties of MALDI-TOF MS can provide a more accurate and effective analysis of the protein.

In the previous study, we purified the Sepharose-unbinding ricin E by using hydroxyapatite chromatography (Woo et al., 1998) and evaluated it as a source for the construction of RTA immunotoxin (Woo et al., 2001). Recently, there have been reports of ricin analysis using CE or mass spectrometry (Hines and Brueggemann, 1994; Despeyroux et al., 2000). However, these studies were restricted to ricin only and there has been no description of the characterization of the ricin components. The aim of this work is to characterize the Sepharose-unbinding ricin E and its subunits by means of two different analytical techniques, i.e. CE and MALDI-TOF MS. As a CE method, sodium dodecyl sulfate–capillary gel electrophoresis (SDS–CGE), which combines the principles of SDS–PAGE with the instrumentation and small diameter capillaries of CE, were used for faster and more efficient separations than SDS–PAGE in the slab gel format (Cohen and Karger, 1987). Since the native toxin and deglycosylated forms are only slightly different in the carbohydrate structure, conventional gel electrophoresis such as SDS–PAGE would not be useful because of insufficient resolution. In this study, the utilization of SDS–CGE has been also described as the most powerful method for identifying deglycosylated ricin (dgRC) and RTA (dgRTA).

2. Materials and methods

2.1. Materials

Castor beans (small grain type) were harvested from the Herbal Garden at SungKyunKwan University. Ricin was purified from the seeds of the castor bean (*R. communis*) by using hydroxyapatite chromatography, as described previously (Woo et al., 1998). The carbohydrates of ricin were chemically modified by treating ricin at pH 3.5 with a mixture of sodium metaperiodate and sodium cyanoborohydride, as described before (Thorpe et al., 1985). RTA, RTB and dgRTA were isolated from reduced and dgRC as described previously (Woo et al., 2001). CE–SDS Protein kit and CE–SDS Protein size standards with molecular weights ranging from 14 to 205 kDa were purchased from Bio-Rad (Hercules, CA, USA). Sinapinic acid was obtained from Sigma (St Louis, MO, USA). All other chemicals used were of analytical reagent grade.

2.2. SDS–CGE analysis

SDS–CGE was carried out using a Bio-Rad BioFocus 3000 CE System with an uncoated fused-silica capillary, 50 μm I.D., 24 cm total length and 19.5 cm to the detector (Bio-Rad). The capillary was rinsed with 0.1 M NaOH, 0.1 M HCl and CE–SDS Run Buffer, for 120, 60, and 240 s, respectively, prior to each injection. To wash out any residual Run Buffer from the outlet surface of the capillary prior to sample injection, two additional purges with CE–SDS Protein Sample Buffer was performed. The samples were prepared and analyzed using the CE–SDS Protein kit (Bio-Rad). An 18 μl of the sample was dissolved in 20 μl of CE–SDS Protein Sample Buffer and 2 μl of CE–SDS Internal Reference (benzoic acid). Samples were heated under non-reducing conditions at 60 °C for 15 min or under

reducing conditions (with 2.5% 2-mercaptoethanol) at 95–100 °C in a water bath for 10 min, cooled on ice for 3 min, and then centrifuged. The injections were carried out using electrophoretic mode (20–40 s at 10 kV). Separations were performed at 15 kV constant voltage and monitored on-column at 220 nm for 12 min. The capillary and sample temperatures were maintained at 20 °C by the liquid cooling system.

2.3. MALDI-TOF MS

MALDI-TOF MS was performed using a Voyager-RP Biospectrometry Workstation (PerSeptive Biosystems, Cambridge, MA). The analysis was conducted in a linear mode and data for 2-ns pulses by the 337 nm nitrogen laser were averaged for each spectrum. Linear, positive-ion TOF detection was performed using an acceleration voltage of 25 kV and a laser intensity approximately 10% greater than the threshold. The grid and guide wire voltages were chosen for each spectrum to achieve the optimal signal to noise. Spectra were obtained by summing over 256 laser shots to obtain the comparable condition and smoothed with a 19-point Savitzky-Golay filter. A saturated solution of sinapinic acid in 70% acetonitrile ultimately containing a concentration of 0.1% trifluoroacetic acid was used as a matrix solution. A mixture of cytochrome C and bovine serum albumin was adapted for the external calibration. The sample–matrix solution was prepared by combining at a ratio of 1:6. Each mixture was thoroughly vortexed and 1 μl of the sample–matrix solution was deposited onto the sample plate and dried by rapid vacuum evaporation.

2.4. SDS–PAGE analysis

SDS–PAGE was performed in the presence of 0.1% SDS using a 10% slab gel (9×8 cm^2). Protein samples were reduced and denatured in 2-mercaptoethanol and SDS at 100 °C, loaded onto the gel, and electrophoresed at 30 mA constant current for approximately 50 min (EC-185, Spectrum, France). The protein bands were visualized with Coomassie brilliant blue staining.

2.5. Concanavalin A Sepharose for determining deglycosylation

A Concanavalin A (Con A) Sepharose affinity column (1.5×15 cm^2) was pre-equilibrated with a 10 mM phosphate buffer containing 0.15 M NaCl (PBS, pH 7.4). The ricin solution, which was treated with a mixture of sodium metaperiodate and sodium cyanoborohydride at pH 3.5, was loaded on the column, and all unbound ricin was washed with the equilibration buffer. Elution of the bound ricin could be achieved using 0.3 M methyl α-D-mannopyranoside in the equilibration buffer. The percentage of the unbound portion was determined to be the quantity of dgRC (Thorpe et al., 1985).

3. Results and discussion

3.1. Characterization of ricin

Fig. 1 shows the SDS–CGE and SDS–PAGE analysis of the ricin purified from the seeds of the castor bean (*R. communis*). Under non-reducing conditions, two partially separated peaks of ricin were detected in the SDS–CGE analysis, whereas only a single band appeared in the SDS–PAGE gel. In the case of native ricin, the A and B chains of the toxin are still linked by an inter-chain disulfide bridge in denatured state. RTA consists of two forms of RTA_1 and RTA_2 with identical protein sequences but with different molecular weights depending upon their differences in carbohydrate content (Trown et al., 1991). Accordingly, ricin is a mixture of two different molecules, $RTB–RTA_1$ and $RTB–RTA_2$. Based on their molecular

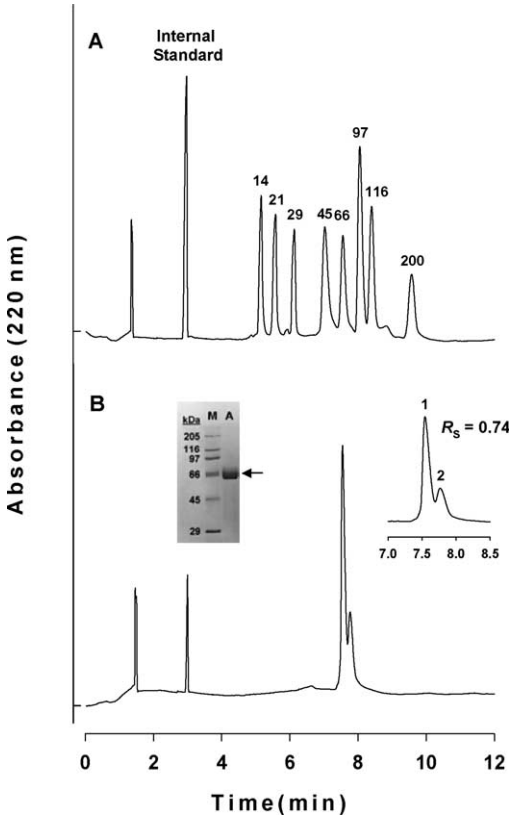

Fig. 1. SDS–CGE and SDS–PAGE (inset) analysis of ricin (A: molecular weight standard marker (14–205 kDa), B: ricin under non-reducing conditions). The right inset is an enlargement of ricin peaks in SDS–CGE. In SDS–PAGE (left inset), arrow represents band corresponding to ricin. SDS–CGE analysis was performed under non-reducing conditions (samples were heated at 60 °C for 15 min without treatment of 2-mercaptoethanol), and internal standard (IS) was benzoic acid. In SDS–CGE, the two peaks of ricin were partially separated ($R_S = 0.74$), whereas only a single band was shown in the SDS–PAGE gel.

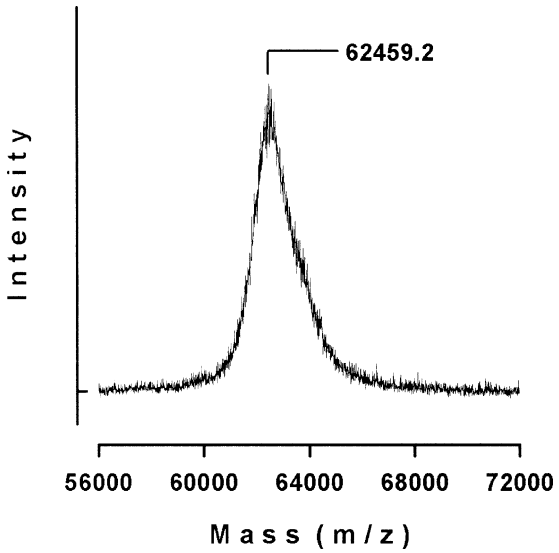

Fig. 2. MALDI-TOF mass spectrum of ricin. The mass number on the peak is the centroided mass-to-charge ratio values of the corresponding peak. A saturated solution of sinapinic acid in water:acetonitrile (70:30, 0.1% TFA) was used as a matrix solution. The sample–matrix solution was prepared by combining at the ratio of 1:6. A mixture of cytochrome C and bovine serum albumin was adapted for the external calibration.

masses, the first major peak was identified as the ricin composed of RTA_1 and RTB, and the second minor peak corresponded to the ricin composed of RTA_2 and RTB. The resolution (R_S) between the two peaks was 0.74. The molecular weight of ricin was determined to be 62,459.2 by MALDI-TOF MS, and the peak was not resolved (Fig. 2).

3.2. SDS–CGE of ricin subunits

Ricin was denatured in the presence of 2-mercaptoethanol under reducing conditions and analyzed by SDS–CGE in the capillary and SDS–PAGE in the slab gel, respectively (Fig. 3A). SDS–CGE exhibited an excellent resolution showing three peaks, while SDS–PAGE provided only two bands, which might be due to the breakage of the disulfide bond resulting in the separation of chains A and B. When compared with electropherograms of the purified subunits (Fig. 3B and C), the first and second peaks in Fig. 3A were identified as peaks from RTA_1 and RTA_2, respectively, and the third peak was identified as RTB. Two peaks of RTA_1 and RTA_2 were resolved to the baseline, and the resolution was 1.49. When compared with Fig. 3C, the RTB peak was completely absent in Fig. 3B indicating the removal of the RTB by the chromatography process. In general, the composition ratio of RTA_1/RTA_2 varies from 2:1 to 3:1 (Trown et al., 1991). In this study, based on the peak area, the ratio of RTA_1 and RTA_2 was calculated to be approximately 2.2:1. This was also similar to the ratio of the major (RTB–RTA_1) and minor (RTB–RTA_2) peaks of

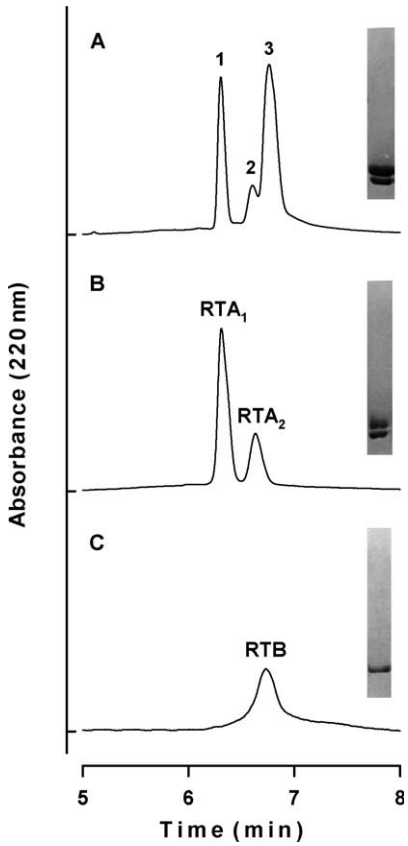

Fig. 3. SDS–CGE and SDS–PAGE (inset) analysis of ricin and its subunits (A: ricin under reducing conditions, B: the purified RTA, C: RTB). Samples were heated under reducing conditions (with 2.5% 2-mercaptoethanol) at 95–100 °C in a water bath for 10 min. SDS–CGE provided an excellent resolution, exhibiting three peaks of reduced ricin (A) and two distinct peaks of RTA ($R_S = 1.49$) (B).

ricin. This confirms that the two peaks of ricin shown in Fig. 1 are likely to be attributable to the difference between RTA_1 and RTA_2 in the carbohydrate composition.

3.3. MALDI-TOF MS of ricin subunits

Fig. 4 represents the MALDI-TOF MS spectra of reduced ricin, RTA, and RTB. As shown in Fig. 4A, the peaks of ricin reduced by 2-mercaptoethanol had masses of 31,005.1 and 31,859.4. The first peak of the mass of 31,005.1 was likely to correspond to RTA_1 shown in the spectrum of RTA (Fig. 4B), but the second peak of mass of 31,859.4 was not consistent with RTA_2 or RTB shown in Fig. 4C. It is considered that the mean values of RTA_2 and RTB were not resolved by MALDI-TOF MS. The molecular masses of the two RTA variants were determined to be 31,059.4 and 32,266.7, respectively (Fig. 4B), and the RTB was measured as 31,533.4 (Fig. 4C).

It is known that the carbohydrate content of RTA_2 is more than double that of RTA_1. Each form possesses a common

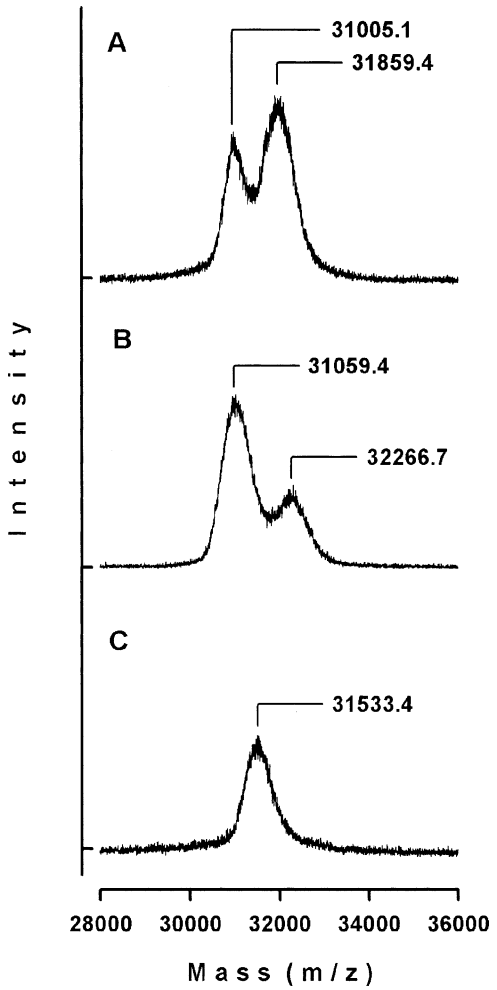

Fig. 4. MALDI-TOF MS of ricin and its subunits (A: ricin under reducing conditions, B: the purified RTA, C: RTB). The analytical conditions were identical to those of MALDI-TOF MS described in Fig. 2. The mass difference (1207 Da) of panel B is almost consistent with mass of the additional carbohydrate content of RTA_2 compared to RTA_1 having single oligosaccharide chain. The molecular mass of RTB, which migrated more slowly than the two isoforms of RTA on SDS–CGE analysis, was *m/z* 31,533.4.

oligosaccharide composed of $(GlcNac)_2(Man)_3XylFuc$ at Asn-10, whereas RTA_2 also possesses an additional high-mannose oligosaccharide, $(GlcNac)_2(Man)_{3\text{-}4}Xyl$, linked to Asn-236 (Trown et al., 1991). Mass difference (1207 Da) between RTA_1 and RTA_2 determined by MALDI-TOF MS is almost consistent with the mass of the additional oligomannose side chain (1204 Da) of RTA_2. This result demonstrates that the greater mass of RTA_2 is solely attributed to its higher carbohydrate content (Foxwell et al., 1985).

3.4. SDS–CGE of deglycosylated forms

The carbohydrates of ricin were chemically modified by treating ricin at pH 3.5 with a mixture of sodium metaperiodate and sodium cyanoborohydride. This resulted in an oxidative cleavage of most of the mannose, fucose and xylose followed by reduction of the aldehyde groups to primary alcohols (Thorpe et al., 1985). The degree of deglycosylation of ricin was estimated to be approximately 60% by Concanavalin A Sepharose affinity chromatography.

Fig. 5 shows the comparison of the SDS–CGE electropherograms of the native toxins and the deglycosylated forms under non-reducing conditions. While the native ricin exhibited two partially resolved peaks (Fig. 5A), the electropherogram of the dgRC showed only one broader peak at a position in some short migration time (Fig. 5B). The dgRTA was purified from the dgRC and identified with SDS–CGE. As shown in Fig. 5D, dgRTA showed broader peaks with faster migration rates as compared with two distinct peaks corresponding to the RTA_1 and RTA_2 (Fig. 5C). The R_S of the dgRTA peaks was lowered to 0.78, while that of RTA was 1.49. As demonstrated above, the lowered resolution of dgRTA might be an indication of deglycosylation of RTA, because the two peaks of native RTA are solely attributed to the difference in the carbohydrate composition. In MALDI-TOF MS, differences in the molecular mass of native toxins and deglycosylated forms were not shown. It is considered that the mass difference may not be in the range of the accuracy of MALDI-TOF MS. The composition ratio between $dgRTA_1$ and $dgRTA_2$ was 2.48:1 similar to that of native RTA_1 and RTA_2. In SDS–CGE measurements, the relative standard deviations (RSD) of migration time for the native toxins and deglycosylated forms were 2.1% or less (data not shown). Because of the high resolution and reproducibility, SDS–CGE may be a useful method for characterizing chemically deglycosylated toxins that exhibit little difference from native toxins based on their molecular masses.

3.5. Molecular mass determination

When analyzed by SDS–CGE and SDS–PAGE, ricin and its subunits migrated at a position, which represents a higher molecular mass than that measured by MALDI-TOF MS. In general, basic proteins and glycoproteins are known to have a decreased charge-to-mass ratio when complexed to SDS, resulting in a decreased migration rate and overestimated molecular mass (Werner et al., 1993). One proposed solution for a more exact estimation of molecular mass in this class of proteins is to use the conditions for a Furguson plot (Guttman and Nolan, 1994). But the Furguson method is time-consuming and not completely correct for mass determination. Since both the A and B chains of ricin are glycosylated, an overestimation on the molecular mass by SDS–CGE and SDS–PAGE was expected. Differences in the measured mass between SDS–CGE and MALDI-TOF MS are shown in Table 1. The difference was larger in more glycosylated forms. In particular, it is noteworthy that the molecular mass of RTB, which migrated more slowly than the two isoforms of RTA on SDS–CGE and SDS–PAGE,

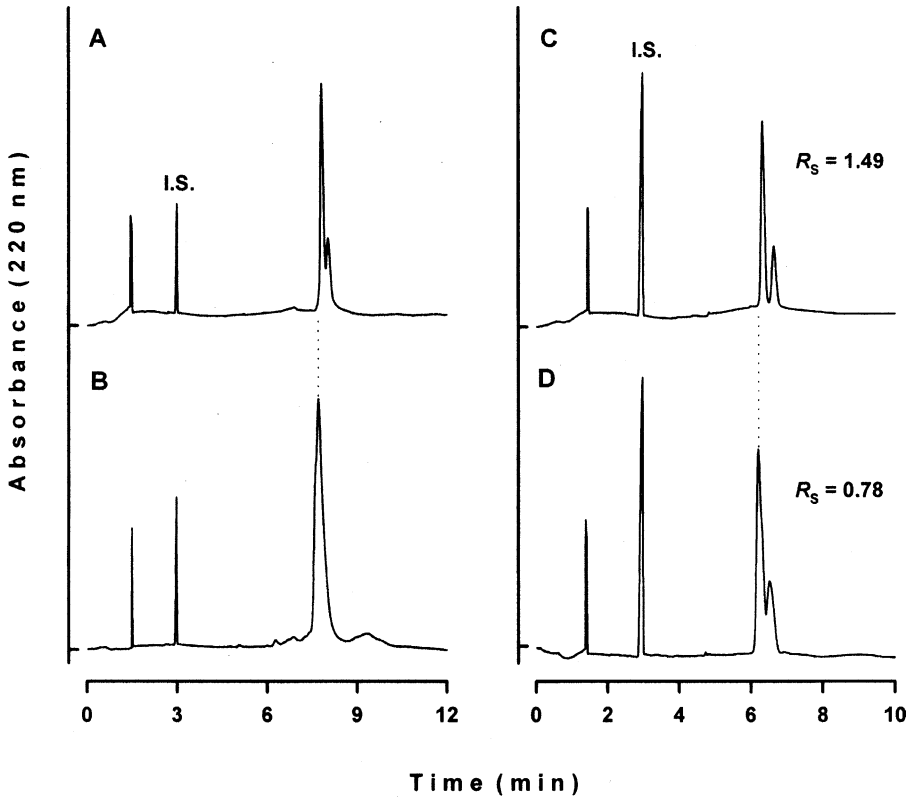

Fig. 5. Characterization of dgRC and dgRTA by SDS–CGE (A: ricin, B: dgRC, C: RTA, and D: dgRTA). The carbohydrates of ricin were chemically modified with a mixture of sodium metaperiodate and sodium cyanoborohydride. Analysis of ricin and dgRC was performed under the non-reducing conditions, and RTA and dgRTA were treated under reducing conditions, followed by SDS–CGE analysis. The single peak of dgRC and the lowered resolution of dgRTA (from $R_S = 1.49–0.78$) represent deglycosylation effect.

was in the middle of RTA_1 and RTA_2 in the measurement of MALDI-TOF MS. Based on SDS–PAGE analysis, the molecular mass of RTB has been generally known to be approximately 34 kDa and the value is higher than those of RTA isoforms (30 kDa for RTA_1 and 32 kDa for RTA_2). This overestimation is likely attributable to the high mannose oligosaccharide present on the RTB. It was reported that the molecular mass of RTB was calculated to be 31,557 Da from the amino acid and the carbohydrate compositions (Araki and Funatsu, 1985). This is almost consistent with the molecular mass measured by MALDI-TOF MS (31,533.4 Da).

4. Conclusions

Ricin and its subunits were characterized by using SDS–CGE and MALDI-TOF MS. This study demonstrated that a more detailed characterization of ricin and its subunits could be possible by combining the high resolution of CE and accuracy of MALDI-TOF MS. As both subunits of ricin are

Table 1
Comparison of SDS–CGE and MALDI-TOF MS in the determination of molecular mass

	SDS–CGE (M_r)[a]	MALDI-TOF MS (m/z)[b]
Ricin (RTA_2–RTB)	92,700	62,459
Ricin (RTA_1–RTB)	83,200	
dgRC	77,100	–
RTB	46,400	31,533
RTA_2	42,900	32,267
$dgRTA_2$	41,300	–
RTA_1	34,100	31,059
$dgRTA_1$	32,900	–

[a] The molecular mass was determined by calibration curve constructed by using SDS–CGE analysis of eight standard protein mixture, shown in Fig. 1A. The calibration curve was constructed by plotting the logarithms of the molecular masses as a function of the electrophoretic mobility.

[b] The represented masses were those of intact samples determined by MALDI-TOF MS and the mixture of cytochrome C and bovine serum albumin was used for the external calibration.

glycosylated, SDS–CGE resulted in an overestimation of the molecular mass. However, the technique exhibited high resolution over other analytical methods due to the presence of carbohydrates in ricin and its subunits. Furthermore, the carbohydrate-induced high resolution of SDS–CGE was useful for differentiating native ricin and RTA from their chemically deglycosylated forms. Also, the exact molecular masses were measured by MALDI-TOF MS and were almost consistent with the expected values. The results shown in this study clearly illustrates the usefulness and necessity of complementary use of two powerful analytical techniques used to characterize ricin and its subunits. Because the methods used in this study are speedy and require only extremely small sample volumes, they may have a potential use in monitoring the production and stability of immunotoxins as well as toxins themselves.

Acknowledgements

This work was supported by the Biotechnology Development Program at the Center for Biologics Evaluation of KFDA (KFDA-03092-LIF-003).

References

Araki, T., Funatsu, G., 1985. Revised amino acid sequence of the B-chain of ricin D due to loss of tryptophan in the cyanogen bromide cleavage. FEBS Lett. 191, 121–124.

Blakey, D.C., Watson, G.J., Knowles, P.P., Thorpe, P.E., 1987. Effect of chemical deglycosylation of ricin A-chain on the in vivo fate and cytotoxic activity of an immunotoxin composed of ricin A-chain and anti-Thy 1.1 antibody. Cancer Res. 47, 947–952.

Cohen, A.S., Karger, B.L., 1987. High-performance sodium dodecyl sulfate polyacrylamide gel capillary electrophoresis of peptides and proteins. J. Chromatogr. 397, 409–417.

Denton, K.A., Tate, S.A., 1997. Capillary electrophoresis of recombinant proteins. J. Chromatogr. B 697, 111–121.

Despeyroux, D., Walker, N., Pearce, M., Fisher, M., McDonnell, M., Bailey, S.C., Griffiths, G.D., Watts, P., 2000. Characterization of ricin heterogeneity by electrospray mass spectrometry, capillary electrophoresis, and resonant mirror. Anal. Biochem. 279, 23–36.

Fenselau, C., 1997. MALDI MS and strategies for protein analysis. Anal. Chem., 661A–665A.

Foxwell, B.M.J., Donovan, T.A., Thorpe, P.E., Wilson, G., 1985. The removal of carbohydrates from ricin with endoglycosidases H, F and D and mannosidase. Biochim. Biophys. Acta 840, 193–203.

Guttman, A., Nolan, J., 1994. Comparison of the separation of proteins by sodium dodecyl sulfate–slab gel electrophoresis and capillary sodium dodecyl sulfate–gel electrophoresis. Anal. Biochem. 221, 285–289.

Hines, H.B., Brueggemann, E.E., 1994. Factors affecting the capillary electrophoresis of ricin, a toxic glycoprotein. J. Chromatogr. A 670, 199–208.

Hunt, G., Nashabeh, W., 1999. Capillary electrophoresis sodium dodecyl sulfate nongel sieving analysis of a therapeutic recombinant monoclonal antibody: a biotechnology perspective. Anal. Chem. 71, 2390–2397.

Karger, B.L., Cohen, A.S., Guttman, A., 1989. High-performance capillary electrophoresis in the biological sciences. J. Chromatogr. 492, 585–614.

Karger, B.L., Chu, Y.-H., Foret, F., 1995. Capillary electrophoresis of proteins and nucleic acids. Annu. Rev. Biophys. Biomol. Struct. 24, 579–610.

Kaufmann, R., 1995. Matrix-assisted laser desorption ionization (MALDI) mass spectrometry: a novel analytical tool in molecular biology and biotechnology. J. Biotechnol. 41, 155–175.

Kreitman, R.J., Pastan, I., 1998. Immunotoxins for targeted cancer therapy. Adv. Drug Del. Rev. 31, 53–88.

Lord, J.M., Roberts, L.M., Robertus, J.D., 1994. Ricin: structure, mode of action, and some current applications. FASEB J. 8, 8201–8208.

Nguyen, D.N., Becker, G.W., Riggin, R.M., 1995. Protein mass spectrometry: applications to analytical biotechnology. J. Chromatogr. A 705, 21–45.

Thorpe, P.E., Detere, S.I., Foxwell, B.M.J., Brown, A.F., Skilleter, D.N., Wilson, G., Forrester, A., Stirpe, F., 1985. Modification of the carbohydrate in ricin with metaperidoate–cyanoborohydride mixtures. Eur. J. Biochem. 147, 197–206.

Thorpe, P.E., Wallace, P.M., Knowles, P.P., Relf, M.G., Brown, A.N.F., Watson, G.J., Blakey, D.C., Newell, D.R., 1988. Improved antitumor effects of immunotoxins prepared with deglycosylated ricin A-chain and hindered disulphide linkages. Cancer Res. 48, 6396–6403.

Thrush, G.R., Lark, L.R., Clinchy, B.C., Vitetta, E.S., 1996. Immunotoxins: an update. Annu. Rev. Immunol. 14, 49–71.

Trown, P.W., Reardan, D.T., Carroll, S.F., Stoudemire, J.B., Kawahata, R.T., 1991. Improved pharmacokinetics and tumor localization of immunotoxins constructed with the Mr 30,000 form of ricin A chain. Cancer Res. 51, 4219–4225.

Vitetta, E.S., Thorpe, P.E., Uhr, J.W., 1993. Immunotoxins: magic bullets or misguided missiles? Immunol. Today 14, 252–259.

Werner, W.E., Demorest, D.M., Stevens, J., Wiktorowicz, J.E., 1993. Size-dependent separation of proteins denatured in SDS by capillary electrophoresis using a replaceable sieving matrix. Anal. Biochem. 212, 253–258.

Woo, B.H., Lee, J.T., Lee, K.C., 1998. Purification of sepharose-unbinding ricin from castor beans (*Ricinus communis*) by hydroxyapatite chromatography. Protein Exp. Purif. 13, 150–154.

Woo, B.H., Lee, J.T., Na, D.H., Lee, K.C., 2001. Sepharose-unbinding ricin E as a source for ricin A chain immunotoxin. J. Immunol. Methods 249, 91–98.