No acceleration of 2378-TCDD decomposition in a later phase was observed. The major decomposition products found were again lower chlorinated dioxins indicating that the dechlorination follows the same route as with pure 2378-TCDD in hexane solution.

These studies document that gamma irradiation from a commercial ^{60}Co-facility can be successfully used for the decomposition of 2378-TCDD and other hazardous compounds. In our study we showed that decontamination of laboratory wastes and contaminated soil was achieved.

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Improved separation at low temperature of glycoproteins by Con A-Sepharose affinity chromatography in the presence of sodium dodecyl sulfate (SDS)[1]

S. Aono, H. Sato, R. Semba and S. Kashiwamata

Department of Perinatology, Institute for Developmental Research, Aichi Prefecture Colony, Kasugai, Aichi 480-03 (Japan),
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Summary. The Con A-Sepharose affinity chromatography of glycoproteins was even more effective at 4 °C than that at room temperature (26–28 °C) in the presence of sodium dodecyl sulfate (SDS). Application of this methodology to the separation of several glycoproteins from SDS-solubilized membrane proteins in rat cerebellum, including a glycoprotein characteristic of the Purkinje cells, was successful.
Key words. Con A-Sepharose; SDS; glycoproteins; cerebellar proteins; affinity chromatography; purification.

Affinity chromatography using immobilized lectins has been used for the fractionation and purification of glycoproteins in biological membranes. While membrane proteins can be readily solubilized by an anionic detergent, sodium dodecyl sulfate (SDS), the detergent has been reported to have a deleterious effect on the affinity for glycoproteins of lectin-Sepharose[2]. In the present study, we investigated effective conditions for purification of glycoproteins by Concanavalin A (Con A)-Sepharose in the presence of SDS. In addition, we tried to separate the GR-250 protein, a Con A-binding glycoprotein[3], with an apparent mol. wt of 250,000[4] from rat cerebellar membrane proteins using the improved method described here.

Materials and methods. Ovalbumin (Sigma, grade III), horseradish peroxidase (Toyobo, Japan) and fetuin from fetal calf serum (Sigma, type III) were used as test materials. Prior to the present study, the commercial preparations of three glycoproteins were checked for their adsorbability on a Con A-Sepharose (Pharmacia) column in the absence of SDS at room temperature. It was shown that peroxidase was completely adsorbed to the column, while ovalbumin and fetuin contained nonadsorbed fractions in their preparations. Thus, the latter two proteins adsorbed to the column were eluted with methyl-α-D-mannopyranoside, dialyzed against 0.1 M Tris-HCl buffer, pH 7.2 (buffer A), and served for later experiments as purified glycoproteins. Peroxidase was used without purification.

Cerebellar samples were prepared at 4 °C unless otherwise stated. Cerebella from 20-day-old Sprague-Dawley strain rats were homogenized in 9 volumes of 10 mM Tris-HCl buffer, pH 7.2. The homogenate was centrifuged at 105,000 × g for 60 min and the resultant precipitate was rehomogenized in one original volume of the same buffer, followed by the addition to 8 volumes of precooled 10 mM Tris-HCl buffer, pH 7.2, containing 0.44 M NaCl and 0.55% (w/v) Triton X-100. After being stirred for 60 min, the suspension was centrifuged again at 105,000 × g for 60 min. The pellet was suspended in the initial buffer and mixed with an equal volume of sample solubilizing solution consisting of 6% SDS; 20% glycerol, 2% 2-mercaptoethanol, and 0.125 M Tris-HCl buffer, pH 6.8, and then boiled for 3 min. The sample was dialyzed against buffer A containing 0.08% SDS and then subjected to Con A-Sepharose column chromatography.

Affinity chromatography of ovalbumin and peroxidase on Con A-Sepharose in the absence of SDS was carried out at room temperature (26–28 °C) or 4 °C. A Sepacol Mini column (Seikagaku Kogyo, Japan) packed with Con A-Sepharose (total volume, 2.5 ml; 0.75 × 5.7 cm) was washed with 5 bed volumes of 0.1 M Tris-HCl-0.02 M sodium borate buffer, pH 7.2 (buffer B), and then of buffer A. After 1 ml of each sample (2 mg/ml) was applied, the column was washed with 5 bed volumes of buffer A. The flow rate was 16 ml/h for ovalbumin and 4 ml/h for peroxidase. The proteins bound to Con A-Sepharose were eluted with buffer B containing 0.2 M methyl-α-D-mannopyranoside at a rate of 3 ml/h.

Affinity chromatography of three test glycoproteins in the presence of SDS was performed under the same conditions as were used in its absence, except for the following modifications. Con A-Sepharose columns were washed with 10 bed volumes of buffer B containing 0.08% SDS (SDS-buffer B) and then with the same volume of buffer A containing 0.08% SDS (SDS-buffer A) to remove loosely bound Con A. Further washing was carried out with 7.5 bed volumes of SDS-buffer A at flow rates

Percent recovery of glycoproteins from Con A-Sepharose column

		SDS (−) 26–28 °C (%)	4 °C (%)	SDS (+) 26–28 °C (%)	4 °C (%)
Ovalbumin	Not adsorbed	0	0	63 ± 6	0
	Adsorbed	105 ± 6	84 ± 3	19 ± 3	83 ± 3
	Total	105 ± 6	84 ± 3	82 ± 3	83 ± 3
Horseradish peroxidase	Not adsorbed	0	0	82 ± 3	0
	Adsorbed	92 ± 1	83 ± 3	7 ± 2	77 ± 1
	Total	92 ± 1	83 ± 3	88 ± 3	77 ± 1
Fetuin	Not adsorbed	–*	–*	58 ± 11	23 ± 8
	Adsorbed	–	–	6 ± 1	56 ± 8
	Total	–	–	64 ± 11	79 ± 2

Each value represents the mean ± SD of three experiments. *Not determined.

of 16, 16 and 4 ml/h before chromatography of ovalbumin, fetuin and peroxidase, respectively. After 1 ml of each sample (2 mg/ml) was applied, the column was washed with 5 bed volumes of the same buffer at the flow rate specified above. Bound proteins were eluted with SDS-buffer B containing 0.2 M methyl-α-D-mannopyranoside (flow rates, 3 ml/h for ovalbumin and peroxidase and 2 ml/h for fetuin). In the cases of ovalbumin and fetuin, Con A was released from the column probably due to a marked decrease in flow rate (from 16 to 3 or 2 ml/h) and interfered with the measurement of proteins in the eluates. To avoid this, ovalbumin and fetuin were eluted after the columns were further washed with 7.5 bed volumes of SDS-buffer A at rates of 3 and 2 ml/h, respectively. Affinity chromatography of cerebellar proteins in the presence of SDS was carried out under the same conditions as that for ovalbumin except for the amount of protein applied (about 6.5 mg). Although the solubility of SDS decreases with decreasing temperature, no precipitation of the detergent was observed under the experimental conditions used. Recoveries of test glycoproteins in the presence and absence of SDS were estimated from the absorbance at 280 nm for ovalbumin and fetuin and at 403nm of the Soret band for peroxidase. A Con A-Sepharose column was freshly prepared in each experiment.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a 9% gel was carried out according to the method of Laemmli[5]. Slab gels were silver-stained following the method of Oakley et al.[6]. Cerebral homogenate for SDS-PAGE was prepared in the same manner as for cerebellar homogenate. The mol.wt estimation was made using mol.wt calibration proteins (Pharmacia; thyroglobulin, 330,000; ferritin, 220,000 for half of the native protein; bovine serum albumin, 67,000; catalase, 60,000; ovalbumin, 43,000; lactate dehydrogenase, 36,000; carbonic anhydrase, 30,000).

Results. In the absence of SDS almost all of the ovalbumin (fig. 1, A and C) and peroxidase (fig. 2, A and C) were adsorbed to the Con A-Sepharose column both at room temperature and at 4 °C. As shown in the table, the total recoveries of both proteins at 4 °C were lower than those at room temperature. Since a greater part of the peroxidase passed through the column when the chromatography was carried out under the same conditions as for ovalbumin (flow rate, 16 ml/h; not shown), the interaction of peroxidase with Con A seems to be weak.

Chromatographic behavior of three test glycoproteins in the presence of SDS differed markedly between the two temperatures, as summarized in the table. At room temperature, a large part of the ovalbumin (fig. 1, B) and peroxidase (fig. 2, B) passed through the column (fraction numbers of 5 to 7) and only a small part was adsorbed on Con A-Sepharose. This was also true of fetuin (elution profile not shown), although the total recovery was considerably lower than those of the other two (table) pre-

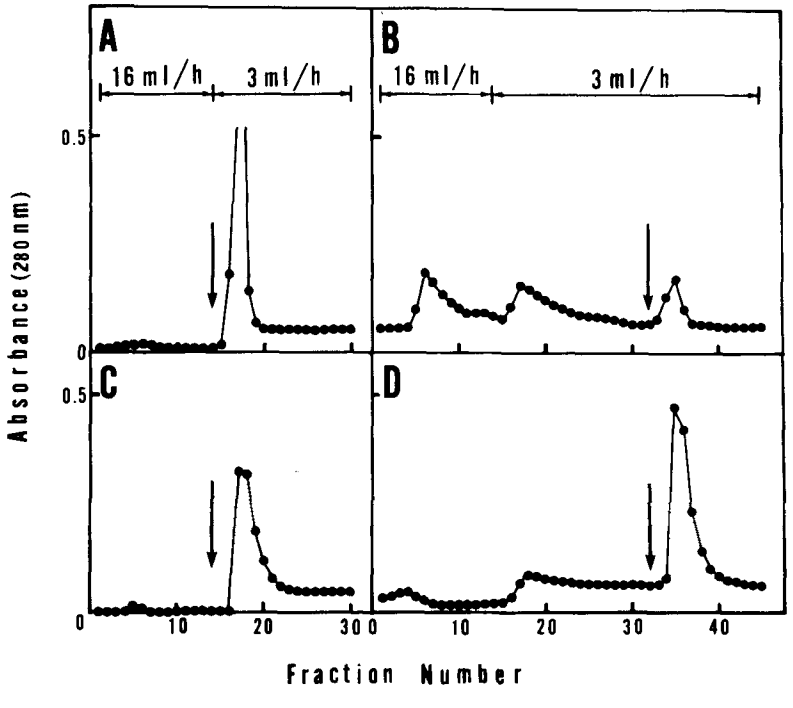

Figure 1. Affinity chromatography of ovalbumin on Con A-Sepharose. About 2 mg of the protein (2 mg/ml) were loaded onto a 0.75 × 5.7 cm column. Elution was conducted initially with buffer A or SDS-buffer A, and at a point indicated by arrow 0.2 M methyl-α-D-mannopyranoside was applied. 1-ml fractions were collected. Protein was measured by the absorbance at 280 nm. For details refer to 'Materials and methods'. A and C, in the absence of SDS; B and D, in the presence of SDS; A and B, at room temperature; C and D, at 4 °C.

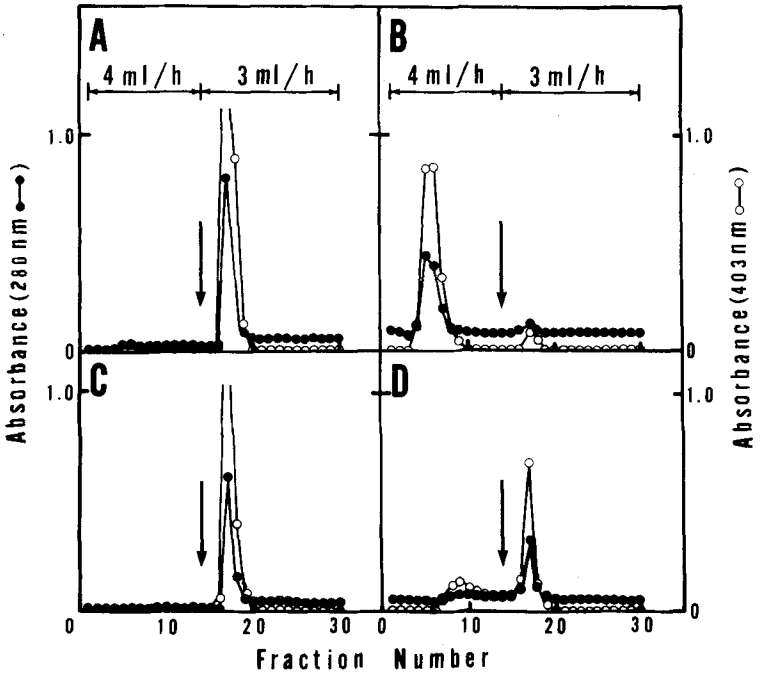

Figure 2. Affinity chromatography of peroxidase on Con A-Sepharose. Experiments were carried out under the same conditions as in figure 1, except for the initial flow rate and the washing procedure with SDS-buffer A (see 'Materials and methods'). Protein was measured by the absorbance at 280 (●—●) and 403 (○—○) nm. A and C, in the absence of SDS; B and D, in the presence of SDS; A and B, at room temperature; C and D, at 4 °C.

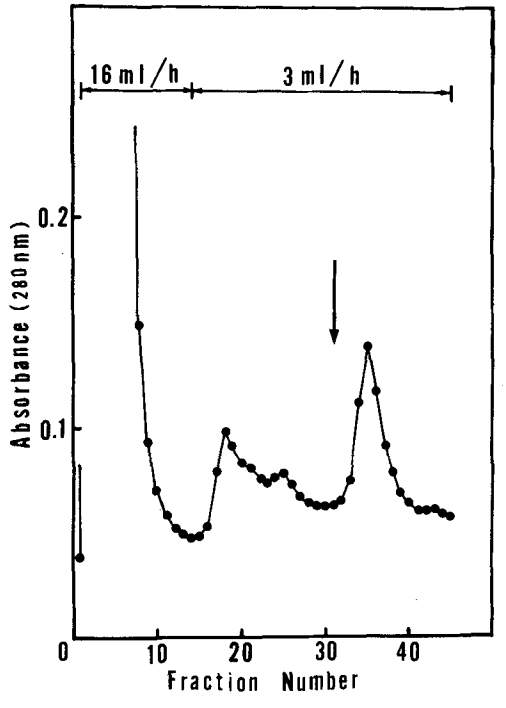

Figure 3. Purification of cerebellar membrane glycoproteins by affinity chromatography on Con A-Sepharose in the presence of SDS at 4 °C. About 6.5 mg of protein (4.3 mg/ml) were loaded onto a column (0.75 × 5.7 cm). The experimental conditions were the same as in figure 1, D. Protein was measured by the absorbance at 280 nm. For details refer to 'Materials and methods'.

sumably due at least in part to the nonspecific binding of the protein to Sepharose matrices. The amount adsorbed relative to the total recovery was found to be higher in ovalbumin (about 23% in average) and far lower in peroxidase (8%) and fetuin (9%). At 4 °C, nearly all the ovalbumin was adsorbed to the column (fig. 1, D), but a small part of the peroxidase was eluted from the column before the addition of mannopyranoside (fraction numbers of 7 to 11 in fig. 2, D). As the latter phenomenon was not observed when a longer column (0.9 × 12.5 cm) was used, the first peak in the profile (fig. 2, D) was regarded as peroxidase adsorbed to the column (table). A conspicuous improvement both in the amount adsorbed and total recovery of fetuin was also achieved at 4 °C, although about a quarter of the protein initially applied still passed through the column (table). The following experiments were carried out to investigate the effect of SDS on the binding capacity of Con A-Sepharose using ovalbumin and peroxidase. A column prepared for affinity chromatography in the presence of SDS at room temperature was washed, to eliminate SDS, with 5 bed volumes of buffer A and then of buffer B containing 1 mM each of $CaCl_2$, $MgCl_2$, and $MnCl_2$. The column was left to stand overnight and used for affinity chromatography in the absence of SDS at room temperature (not shown). Most of the ovalbumin was adsorbed to the column. The chromatographic pattern of peroxidase was almost the same as that at 4 °C in the presence of SDS, although the first peak (refer to fig. 2, D) was more retarded. These results indicate that the binding capacity of the SDS-treated column is well preserved.

Purification of glycoproteins from cerebellar membrane proteins in the presence of SDS was attempted with use of the methodology described above. After extraction of the cerebellar particulate fraction with 0.5% Triton X-100 in final concentration, 32.1 ± 4.7% (n = 8) of the homogenate proteins remained insoluble. Upon Con A-Sepharose affinity chromatography of the Triton X-100 insoluble proteins, 87.0 ± 6.0% (n = 3) of the proteins were not bound. The rest were adsorbed and 1.9 ± 0.2% (n = 3) were recovered from the Con A-Sepharose column (fig. 3). The electrophoretic profiles of the eluted proteins and cerebellar homogenate as a reference are shown in figure 4. As seen in figure 4, A, distinctly recognizable were six protein bands (a–f). Their apparent mol.wts were estimated using the standard proteins (fig. 5). Among them a fairly concentrated 250,000 mol. wt band (band a in figs 4, A, and 5) was found to coincide in mobility with a protein indicated by an arrowhead in the electrophoretogram of cerebellar homogenate (fig. 4, B, and also see fig. 5).

Discussion. Con A-Sepharose affinity chromatography is commonly carried out at room temperature. The amounts of ovalbumin and peroxidase recovered from the columns at room tem-

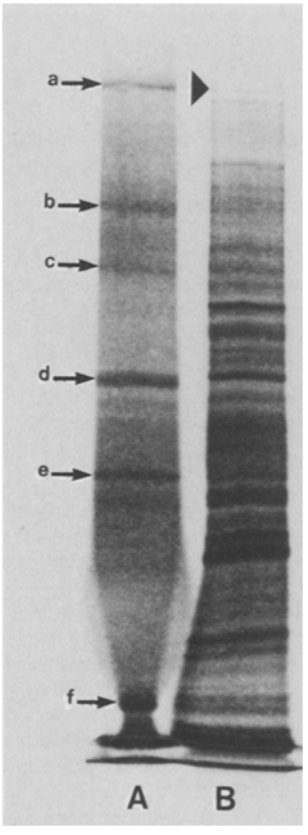

Figure 4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of rat cerebellar proteins. The concentration of acrylamide was 9%. The slab gel was silver-stained. *A* glycoproteins purified from the 0.5% Triton X-100 insoluble fraction by affinity chromatography on Con A-Sepharose (11.6 µg/90 µl). Six distinct protein bands (a–f) are shown. *B* cerebellar homogenate (9.9 µg/20 µl). Arrowhead indicates a protein with an apparent mol.wt of 250,000 (refer to fig. 5).

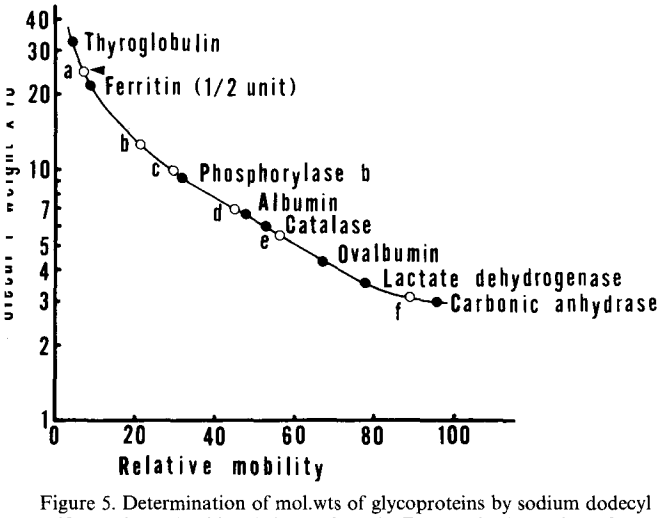

Figure 5. Determination of mol.wts of glycoproteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. For standard proteins refer to 'Materials and methods'. a–f and arrowhead correspond to the protein bands shown in figure 4, A and B, respectively. The apparent mol.wts estimated are: a and arrowhead, 250,000; b, 130,000; c, 100,000; d, 71,000; e, 56,000; f, 31,000.

perature were actually higher than those at 4 °C in the absence of SDS (table). In the presence of SDS, however, the affinity chromatography of glycoproteins on Con A-Sepharose gave much better results at 4 °C than at room temperature: The amount of test glycoproteins bound to and eluted by mannopyranoside from the Con A-Sepharose column at room temperature was at its highest only 19% (ovalbumin; table), while a sufficient amount of Con A remained in the column (see text). These results coincided with the report of Lotan et al.[2], who showed that in the presence of 0.1% SDS at 23 °C, only 23% of Con A was dissociated into subunits and released from the column after a prolonged period of incubation (16 h), but the glycoprotein (asialo[^{3}H]-fetuin) binding efficiency was reduced to 18% of the SDS-free control. At 4 °C, even though SDS was present, most of the ovalbumin and peroxidase and more than half of the fetuin were adsorbed on Con A-Sepharose and recovered from the column. The findings appear to suggest that the interaction of Con A with the glycoproteins at 4 °C is different from that at room temperature in the presence of SDS.

Our aim is the isolation and purification from the SDS-solubilized rat cerebellar particulate fraction of the GR-250 protein, which is a Con A-binding glycoprotein markedly reduced in association with cerebellar hypoplasia in jaundiced Gunn rats[3,4] and is identified as the P_{400} protein[3] characteristic of cerebellar Purkinje cells[7]. A protein band with an apparent molecular weight of 250,000 shown in the electrophoretogram of cerebellar homogenate (fig. 4, B, arrowhead) was also found concentrated in the eluate from the Con A-Sepharose column (band a in fig. 4, A). A protein with the same mol.wt was detected as well in an even smaller amount in cerebral homogenate (not shown), which is consistent with the previous observations[3]. Judging from the electrophoretic behavior, mol.wt, Con A-binding and abundance in the cerebellum, these proteins could be regarded as the GR-250 protein, though this is not conclusive. Thus, the use of Con A affinity chromatography in the presence of SDS at 4 °C is considered to be effective in the purification and concentration of the GR-250 protein without causing any change in mol.wt. Besides this protein, several other Con A-binding glycoproteins were also detected in the electrophoretogram of the Con A-Sepharose column eluate (fig. 4, A). Although the adsorbability of glycoproteins on Con A-Sepharose and their recovery in the eluate may differ from each other as shown in the cases of test proteins, the present method is thought to be useful for the isolation of glycoproteins which possess affinity for Con A and are solubilized by SDS.

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