[Regular Paper]

Marked suppression of LDH activity by the sugar chain of LDH-binding immunoglobulin G in the serum of a patient with Myocardial infarction

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SUMMARY

We encountered a very rare case of cardiac failure in which serum-negligible LDH activity was detected. The patient was a 58-year-old male with a history of myocardial infarction. In this case, LDH was bound to the lambda type of immunoglobulin G; and the activity of both subunit's of LDH, i. e., LDH-H (B) and LDH-M (A), were suppressed. As we reported formerly, this suppression of LDH activity is considered to be attributable to the binding of immunoglobulin G. In our previous study, we analyzed the structure of the sugar chain of the binding immunoglobulin G (IgG) after treatment with various enzymes, and found that the binding capacity of the IgG in the patient's serum to LDH isozymes was changed by treatment with neuraminidase. In the present study, we removed the sialic acid residue from the IgG in the patient's serum by means of neuraminidase digestion, and thereafter attempted to ligate the resultant asialo-immunoglobulin G to LDH isozymes from normal volunteers in order to clarify the mechanism of the suppression of LDH isozymes. We found that the antigenicity of the IgG in the patient's serum was reduced by removing its sialic acid residue. These findings demonstrate that the sialic acid residue of the sugar chain of the IgG in the patient's serum was directly associated with the suppression of LDH activity by the enzyme-binding immunoglobulin observed in this case of cardiac failure with a history of myocardial infarction.

Key words: LDH linked immunoglobulin, LDH anomaly, LDH isozyme, sialic acid, sugar chain.

INTRODUCTION

We encountered a very rare case of cardiac failure. In this patient, who had a history of myocardial infarction hardly any LDH activity was detected in the serum. It is known that suppression

of LDH activity is sometimes caused by the binding of an autogenous immunoglobulin to LDH (frequently the binding of immunoglobulin A to LDH-M (A))¹⁾.

Analytical findings in this case reveal that the activity of both subunit's of LDH, i. e., LDH-H (B)

心筋梗塞患者血清にみられた LDH 結合性免疫グロブリン G の糖鎖による著しい LDH 阻害機序に関する新知見

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and LDH-M (A), was suppressed by the lambda type of immunoglobulin G,²⁾ and that the sugar chain moiety of the binding immunoglobulin was involved in this suppression.³⁾ In the present study, we have attempted to clarify the mechanism of the suppression of LDH activity by enzyme-binding immunoglobulin at the level of the sugar chain in this IgG.

The results show that the sialic acid residue of the sugar chain in the IgG in the patient's serum suppressed the activity of LDH isozymes obtained from healthy volunteers, and that the antigenicity

of the IgG in the patient's serum was reduced by removal of the sialic acid residue from the sugar chain. These results suggest strongly that the sialic acid residue of the sugar chain in the IgG is closely associated with the mechanism of suppression of LDH activity.

CASE

The patient was a 58-year-old male with cardiac failure who had a history of myocardial infarction.²⁾

In this case, LDH activity could not be detected

Fig. 1. Preparation of the samples.

According to the procedure shown in Fig. 1-A, LDH and LDH-IgG complex are absorbed by affinity chromatography using 5'-AMP-sepharose 4B. Then, free IgG is obtained in the effluent. Free IgG is then separated and purified by ion-exchange chromatography using a DEAE-Sephacel, followed by neuraminidase digestion according to procedure 1 of Fig. 1-B. The protein (i.e., IgG after removal of the sialic acid residue by neuraminidase digestion) in the fraction is absorbed with protein A-Sepharose Cl 4B, and the sugar chain moiety (the sialic acid residue removed from IgG) is eluted. The absorbed protein is eluted in glycine-HCl buffer (pH 3.0). In this way, IgG is divided into the protein fraction and the sugar chain moiety. The sugar chain moiety is lyophilized. The protein fraction is concentrated, and a part of this portion is treated with beta-galactosidase according to procedure 2 of Fig. 1-B. A similar treatment is made with beta-N-acetylglucosaminidase or endo-beta-N-acetylglucosaminidase D to separate the protein fraction and the sugar chain moiety of IgG. The protein fraction is tested without further processing, while the sugar chain moiety is lyophilized. The lyophilized sugar chain moiety is thawed in distilled water before it is used as a test specimen.

Fig. 2. Glycosidic bonds at which sugar chain moiety was cleaved with glycosidases.¹¹⁾

because LDH isozymes were predominantly bound to the lambda type of IgG. In a closer analysis, both subunit's of LDH, i. e., LDH-H(B) and LDH-M(A), were found to have been suppressed because they were bound to the IgG at a location closely related to the substrate binding site. LDH activity in the erythrocyte, however, was shown to be normal.

METHODS

Isolation and purification of LDH isozymes and IgG

LDH isozymes were isolated and purified according to the method of Bruce et al.⁴⁾ and Igarashi et al.⁵⁾ Free IgG, which is not involved in the patient's LDH-IgG complex, was isolated from the patient's serum utilizing the mini-column method of Ishiguro et al.⁶⁾ (Fig. 1-A).

Determination of LDH isozymes

LDH isozymes were determined by agarose-gel electrophoresis.⁷⁾

Glycosidase digestion⁸⁾ (Figs. 1-B and 2)

(1) Neuraminidase digestion

Neuraminidase (EC 3. 2. 2. 18),^{9,10)} IgG fraction (isolated by the method described above) and 0. 25 M acetic acid buffer (pH 6. 5) were mixed at a ratio of 2 : 1 : 1 to yield a final neuraminidase concentration of 0. 1 U. This mixture was incubated for 30 h at room temperature.

(2) Beta-galactosidase digestion

10 U (0. 22 u/mg) of lyophilized beta-galactosidase (EC 3. 2. 1. 23) from bovine liver was purchased from Sigma Chemical Co., Ltd. This enzyme was dissolved in 0. 04 M citric acid buffer (pH 6. 0).

The solution was then added to a test specimen (IgG fraction after removal of the sialic acid residue by neuraminidase digestion) to yield a final beta-galactosidase concentration of 0. 1 U. The mixture was then incubated in a thermostat (37°C) for 3 h.

(3) Beta-N-acetylglucosaminidase digestion

Beta-N-acetylglucosaminidase (EC 3. 2. 1. 30)¹¹⁾ from bovine kidney was purchased from Seikagaku Kogyo Co., Ltd. in amounts of 5 mg (0. 9 ml of 3. 2 M ammonium sulfate suspension, pH 6. 0).

This enzyme was diluted to appropriate concentration with 0. 04 M citric acid buffer (pH 6. 0) and then added to a test specimen (IgG fraction after removal of the sugar chain moiety by beta-galactosidase digestion). The mixture was then incubated in a thermostat (37°C) for 3 h.

(4) Endo-beta-N-acetylglucosaminidase D digestion

0. 5 U of endo-beta-N-acetylglucosaminidase D (EC 3. 2. 1. 96)^{12,13)} from *Streptococcus pneumoniae* was purchased in the form of lyophilized powder from Seikagaku Kogyo Co., Ltd. This enzyme was dissolved in 0. 04 M citric acid buffer (pH 6. 0) and then added to a test specimen (IgG fraction after removal of the sugar chain moiety by successive digestions with neuraminidase, beta-galactosidase and beta-N-acetylglucosaminidase) to yield a final endo-beta-N-acetylglucosaminidase D concentration of 0. 1 U. The mixture was then incubated in a thermostat (37°C) for 3 h.

Lyophilization

Using a Kyowa vacuum lyophilizer (model R2L-20 NB, Kyowa Shinkuu Gijutsu K. K.), the sample

was lyophilized for 24 h at -30°C .

Ouchterlony test

Using a anti-human serum produced by DAKO-PATTS Co., Ltd. and purchased from Kyowa Medex Co., Ltd., an Ouchterlony test was carried out according to the micro-Ouchterlony method.^{14,15)}

RESULTS

Because the suppression of LDH activity was observed in this case and seemed to be attributable to the sugar chain moiety of binding immunoglobulin, we studied on the binding immunoglobulin with respect to the correlation between its sugar chain moiety and the enzyme activity, as well as its antigenicity.

Effect of the sugar-chain-removed IgG on the LDH activity of LDH isozymes

After the sugar chain was removed from the

IgG in the patient's serum by treatment with various enzymes, the remaining moiety (protein) of IgG (IgG fraction obtained by the procedure described above) was examined concerning whether the inhibitory effect of IgG on LDH isozymes is changed by the removal of the sugar chain moiety, and which moiety of the IgG enables to suppress LDH activity. This was performed using a method by which the sugar-chain-removed IgG was first mixed with control serum revealing marked activities of LDH isozymes, and then its effect on LDH isozymes was assessed. The results are shown in Fig. 3. When it was attempted to ligate the sugar-chain-removed IgG fraction from normal controls (in whom no LDH-IgG complex was observed) to normal LDH isozymes of the above-mentioned control serum, the LDH zymogram did not differ from that in the case of control LDH isozymes (Fig. 3-A).

Fig. 3. Effect of sugar-chain-removed IgG on LDH isozymes.

(A) The remainder fractions, the protein fractions with no or some sugar chain's, after removal of the sugar chain moiety from normal volunteer's purified IgG by treatment with various glycosidases. (B) The remainder fractions, the protein fraction with no or some sugar chain's, after removal of the sugar chain moiety from patient's purified IgG by treatment with various glycosidases. a : neuraminidase digestion, b : beta-galactosidase digestion, c : beta-N-acetylglucosaminidase digestion, d : endo-beta-N-acetylglucosaminidase D digestion, e : sample, f : control LDH isozyme. Control serum to reveal marked activities of LDH isozymes was to each protein fraction to assess its effect on the LDH isozymes.

Also when the sugar-chain-removed IgG from the patient was added to normal LDH isozymes, no changes in the LDH zymogram were shown when compared with that of normal LDH isozymes (Fig. 3).

Effect of the sugar chain moiety, removed from the IgG, on LDH isozymes

The sugar chain was removed from the IgG by treatment with various enzymes and its effect on LDH isozymes was assessed in order to clarify the relationship between the sugar chain moiety and the inhibitory effect on LDH, and to examine which of these glycosidase treatments allow us to obtain a sugar chain fraction which inhibits LDH. For these purposes, the sugar chain fractions obtained from the IgG by treatment with these glycosidases were added to normal LDH isozymes with marked activities and observed on electrophoresis. The results are shown in Fig. 4. When the sugar

chain fractions isolated from the IgG of normal controls by similar enzyme treatments were added to normal LDH isozymes, the LDH zymogram showed no changes. When the sugar chain fraction isolated from IgG in the patient's serum by treatment with beta-galactosidase, or beta-N-acetylglucosaminidase or endo-beta-N-acetylglucosaminidase D was added to normal LDH isozymes, the LDH zymogram did not differ from that of control LDH isozymes. However, when the sugar chain fraction isolated from IgG in the patient's IgG serum by treatment with neuraminidase was added to normal LDH isozymes, the LDH zymogram showed a pattern of strongly suppressed LDH activity (Fig. 4).

Antigenicity of the sugar-chain-removed IgG fraction from the patient's serum

The IgG in the patient's serum after removal of the sugar chain by treatment with various enzymes

Fig. 4. Effect of the sugar chain obtained from the IgG on LDH isozymes.

(A) The sugar chain moiety of IgG (isolated from normal serum and purified) obtained by glycosidase digestion. (B) The sugar chain moiety of IgG (isolated from patient's serum and purified) obtained by glycosidase digestion. a: neuraminidase digestion, b: beta-galactosidase digestion, c: beta-N-acetylglucosaminidase digestion, d: endo-beta-N-acetylglucosaminidase D digestion, e: sample, f: control LDH isozyme. Control serum to reveal marked activities of LDH isozymes was added to each sugar chain fraction to assess its effect on the LDH isozymes.

Fig. 5. Antigenicity of sugar-chain-removed IgG of the patient.

The IgG, isolated from patient's serum was treated with the following glycosidases; 1: neuraminidase, 2: beta-galactosidase, 3: beta-N-acetylglucosaminidase and 4: endo-beta-N-acetylglucosaminidase D. The sugar-chain-removed IgG (protein fraction) thus obtained was poured into each well. The IgG from the patient's serum was poured into well 5. Distilled water was poured into well 6 to serve as a control. IgG anti-serum, 3 μ l, was poured into the central well. The specimens were incubated for 3 days or more at room temperature.

was examined on its antigenicity using the Ouchterlony test. The results are shown in Fig. 5. The IgG in the patient's serum reacted with anti-IgG serum. When the sugar chain was removed by neuraminidase digestion, the antigenicity of the IgG in the patient's serum was weakened and the antigenicity was further reduced by the removal of the sugar chain by beta-galactosidase. The antigenicity became much lower when the sugar chain was removed by beta-N-acetylglucosaminidase. The antigenicity was not detected any more when the sugar chains of the IgG in the patient's serum were completely removed by endo-beta-N-acetylglucosaminidase D digestion (Fig. 5).

DISCUSSION

The presence of an inhibitory factor against LDH in the serum was first reported by Peel¹⁶⁾ in 1969.

Thereafter, about 10 cases in which inhibitory factors were found against some enzymes, includ-

ing LDH, have been reported. Although the suppression of LDH activity seems to be associated with enzyme-binding immunoglobulin, the mechanism of the inhibition remains to be clarified.

In the present case in which hardly any LDH activity was detected, serum LDH isozymes were bound to immunoglobulin and the IgG was found to be able to bind also to normal LDH isozymes to suppress the enzyme activity, while the activity as well as the pattern of LDH isozymes was shown to be normal in the red cells.²⁾ Based on these results, we speculated that the IgG in the patient's serum rather than the patient's LDH would be abnormal.

The binding activity of the patient's purified IgG to LDH isozymes was changed by treatment of the IgG with neuraminidase, suggesting that the binding of the IgG to LDH isozymes and the suppression of LDH activity by the IgG must be attributable to the sugar chain moiety of the IgG.³⁾

In the present study, the addition of the fraction containing sialic acid residues (isolated from the IgG in the patient's serum by neuraminidase digestion) to normal LDH isozymes resulted in a decrease of LDH activity and the antigenicity of the IgG in the patient's serum was reduced by removal of its sialic acid residues. These results allow us to conclude for the first time that the sialic acid residue of the sugar chain moiety in the IgG in the patient's serum is most closely related to the suppression of LDH activity by the enzyme-binding immunoglobulin from our case. As it is known that N-acetylneuraminic acid is the most common sialic acid in normal individuals, it is presumed that further investigation into the nature of the abnormal IgG with regard to its sialic acid residue is necessary.

In any event, the results of our former and present studies suggest that the abnormality in the sialic acid residue of the IgG sugar chain moiety resulted in the binding of the IgG in the patient's serum to normal LDH isozymes.

In present case, serum LDH activity could not be detected because of binding of the IgG to the LDH isozymes. It remains to be elucidated why LDH

activity can not be detected when IgG binds to LDH isozymes. One possible explanation is that abnormal IgG bound to the LDH isozymes at the region near the active site, as did the substrate, and as a result markedly decreased formation of the enzyme substrate complex occurred. In some cases similar to ours, completely suppressed activity of LDH was shown, and in other cases partially suppressed activity was seen at first and several days later it became completely suppressed. It has so far been considered that LDH activity is strongly suppressed by the blocking of the active site on the enzyme molecule, and that such differences in the inhibitory effects on LDH activity by abnormal IgG depend on the differences in binding affinity to the active center of the enzyme. It has been reported that in elderly individuals abnormal IgGs were frequently appeared and also that an increase in serum IgGs with unknown antigen-specificity was seen in the serum, suggesting an association with the abnormal IgG in this case.

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要 旨

心筋梗塞の既往歴をもつ58歳、男性の心不全患者で、LDH活性がほとんど検出されないまれな症例であった。LDHは免疫グロブリンG、 λ 型と結合し、LDH-H (B)およびLDH-M (A)の両サブユニットとも阻害されていた。この阻害現象は結合性免疫グロブリンGによっておこることをわれわれはすでに明らかにした。また、この阻害機序に関係のある結合性免疫グロブリンG糖鎖の酵素による構造解析を行った。その結果、患者IgGをノイラミニダーゼ酵素処理することによって結合能に変化が認められること、本論文でノイラミニダーゼ酵素処理で切り離した患者シアル酸を他の正常LDHアイソザイムに添加すると阻害がみられること、患者IgGからシアル酸を切り離すことによって抗原性が弱まることから本例の酵素結合性免疫グロブリンGによってLDHが阻害されるものに最も直接的に関与しているものは、患者IgG糖鎖のシアル酸であることが初めて明らかとなった。