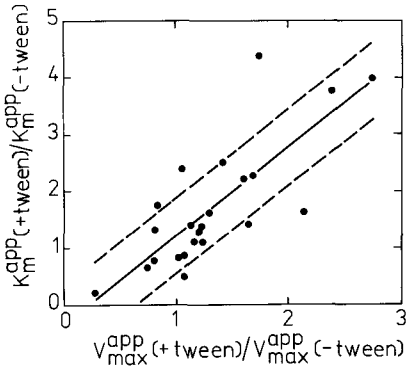

Figure 2. The relationship between the kinetic parameters K_m^{app} and V_{max}^{app} for LPL hydrolysis of the VLDL particles after tween-20 treatment (n = 23). The relation is subordinated to the equation y = 1.54x − 0.34 (r = 0.78). For the native VLDL V_{max}^{app} was equal to 50.5 ± 6.0 µmole H^+/min per mg of LPL protein, K_m^{app} was equal to 0.128 ± 0.036 mg/ml.

tion between apo C-III and the sorbent-immobilized enzyme. Thus, our results suggest an effective interaction between the inhibitor and the enzyme-substrate complex on the particle surface. We may conclude that the apo C-II activator action in the described model experiments is concentration-dependent, and that this behaviour is due to the effect of this apolipoprotein on the lipid phase state of the LPL hydrophobic substrate. This correlates with the observation [14] that in male VLDL, when there is a high HDL cholesterol concentration and a low TG plasma level – indicating that lipolysis is effective – the absolute and relative contents of apo C-II are reduced. In addition, it is suggested that lipolysis in vivo is influenced by the relative surface concentration of apo C-II and apo C-III, the modulators of LPL activity.

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0014-4754/89/050461-03$1.50 + 0.20/0

Purification of α-ketoaldehyde dehydrogenase from the human liver and its possible significance in the control of glycation

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Received 13 October 1988; accepted 17 January 1989

Summary. Alfa-ketoaldehyde dehydrogenase, which was extracted and purified from human livers, may act on carbonyl compounds, such as 3-deoxyglucosone, and be involved in the control of glycation (Maillard reaction) in the body.
Key words. α-Ketoaldehyde dehydrogenase; 3-deoxyglucosone; Maillard reaction; glycation and Diabetes mellitus.

Glycation is the nonspecific binding between an amino group of a protein and a reduced sugar. The process of glycation is sometimes referred to as the Maillard reaction [1]. The early-stage products of the Maillard reaction have been used clinically as an indicator of blood glucose control in diabetic patients [2]. This reaction involves repeated rearrangements and dehydrations after the initial early stage, and generates brown pigments, called late-stage products [3], which contain cross-links [4] and have the ability to fluoresce. Recently, it has been reported that these late-stage products exist in the living body [4–7], and they have been studied in connection with the etiology of diabetic complications and aging [8]. In view of the fact that 3-deoxyglucosone (3-DG) [9, 10] and other carbonyl compounds play an important role in the formation of late-stage products in the Maillard reaction, we attempt-

ed to extract the enzyme α-ketoaldehyde dehydrogenase, which acts on 3-DG[11], and to investigate its mechanism of action.

Materials and methods. Purification of the enzyme was performed according to the method of Ray et al.[12] with some modifications. The liver was removed from a 30-year-old male who underwent autopsy 2 h after death from an accident. The liver was dehematized, perfused and chopped, and then homogenized with acetone at −20 °C to prepare an acetone powder. 10 g of the acetone powder were mixed with 100 ml of 10 mM sodium phosphate buffer, pH 7.2, which contained 1 mM β-mercaptoethanol. After 2 h of stirring at 4 °C, the mixture was centrifuged at 3000 rpm at 4 °C for 30 min. Ammonium sulfate (313 g/l) was added to the supernatant with constant stirring to give 50% saturation. After standing for 30 min, the suspension was centrifuged at 15,000 rpm for 30 min. Ammonium sulfate (137 g/l) was added to the supernatant, with stirring, to give 70% saturation. After another centrifugation, the supernatant was discarded and the precipitate was retained. The precipitate was dissolved in 10 ml of 10 mM sodium phosphate buffer, pH 7.2, which contained 1 mM β-mercaptoethanol.

The solution was dialyzed against a sufficient volume of 20 mM sodium phosphate buffer, pH 7.2, which contained 1 mM β-mercaptoethanol in a seamless cellulose tube (Union Carbide Co., Ltd, New York, U.S.A.) at 4 °C for 7 h for desalting. An Econo-column (Bio-Rad Co., Ltd, Richmond, U.S.A.) was packed with 10 ml of preswollen diethyl-aminoethanol (DEAE) cellulose gel and equilibrated with 10 mM sodium phosphate buffer, pH 7.2, plus β-mercaptoethanol. An aliquot of 10 ml of the desalted extract was loaded onto the DEAE column and eluted with 50 ml of the above-mentioned buffer. Then, an affinity column packed with a bed volume of 5 ml of TSK-GEL AF-Red Toyopearl 650 ML (Toso Co., Ltd, Tokyo, Japan) was connected to a Fast Protein Liquid Chromatography system (Pharmacia Co., Ltd, Uppsala, Sweden). Using a superloop, 20 ml of the eluate of the enzyme extract from the DEAE column was injected. The column was eluted with 10 mM sodium phosphate buffer that contained 1 mM β-mercaptoethanol and with steps of 0.1 M, and 0.15 M KCl in sodium phosphate buffer. The activity was eluted with KCl by the step gradient method. The peak of protein was detected by monitoring the absorbance at 280 nm. The eluted enzyme extract was desalted on a PD-10 column (Pharmacia Co., Ltd, Uppsala, Sweden).

The activity of α-ketoaldehyde dehydrogenase was measured at 340 nm. Assay conditions: 50 μg of extract in 2 ml of 75 mM Tris-HCl buffer (pH 8.6, 30 °C) containing 10 μmol of NAD and 5 μmol of methylglyoxal.

Changes of the enzymatic activity were determined under the same conditions using an enzyme extract incubated with 1 mM para-chloromercuribenzoate (p-CMB) during 60 min at room temperature.

The molecular weight of α-ketoaldehyde dehydrogenase was determined by the sodium dodecyl sulfate (SDS)-gradient polyacrylamide gel electrophoresis (PAGE) method[13] using a gel electrophoresis apparatus, GE-2/4LS (Pharmacia Co. Ltd, Uppsala, Sweden), a 4–30% polyacrylamide gradient gel, and a calibration kit for molecules of low molecular weight (Pharmacia Co., Ltd, Uppsala, Sweden).

In another experiment, four kinds of pretreatments were performed using 15 μmol of 3-DG as substrate, with or without 100 μg of α-ketoaldehyde dehydrogenase, and 10 μmol of NAD in 2 ml of 50 mM sodium phosphate buffer, pH 8.0 for 2 h. Thus, 3-DG was preincubated under the following conditions:

A) α-ketoaldehyde dehydrogenase (−), NAD (+), 30 °C
B) α-ketoaldehyde dehydrogenase (+), NAD (−), 30 °C
C) α-ketoaldehyde dehydrogenase (+), NAD (+), 0 °C
D) α-ketoaldehyde dehydrogenase (+), NAD (+), 30 °C

After preincubation, 1 mg/ml of lysozyme was added. Half of the mixture was then incubated with 3 mM of sodium azide at 37 °C for 14 days and the other half was frozen at − 40 °C. Fluorescence was measured before and after incubation, after the addition of lysozyme, with a spectrophotometer Model 650–60 (Hitachi Co., Ltd, Tokyo, Japan) with an excitation wavelength of 370 nm and an emission wavelength of 440 nm, and was expressed in arbitrary units/mg protein.

Statistical analysis was performed by Student's t-test.

Results. Alfa-ketoaldehyde dehydrogenase was eluted, without being retarded, from the DEAE cellulose gel. As shown by the solid line in figure 1, most of the protein in the extract was not retarded on the affinity column chromatography. However, as shown by the dotted line, the enzymatic activity was found to coincide with the peak of protein eluted with 0.15 M KCl. The specific activity of this eluted sample was 4.65 μmol/mg/min. The specific activity of the enzyme at the stage of final purification was increased about 6000-fold over the crude extract from acetone powder and the overall yield was about 35%.

The purified enzyme, after affinity chromatography and desalination, reacted with methylglyoxal and 3-DG as substrates, in the presence of NAD, with Km values of 0.24 mM and 0.47 mM, respectively. The addition of 1 mM p-CMB to the former reaction system at room temperature resulted in almost complete loss of enzyme activity (fig. 2). The enzyme was estimated to have a molecular weight of 43 kDa by SDS-PAGE (fig. 3).

The fluorescent intensity which was produced after incubation with lysozyme at 37 °C for 14 days was significantly higher in the enzyme free-(group A) or the NAD-free (group B) preincubation groups than in the enzyme-added preincubation groups (C and D) and lower in the enzyme-added 30 °C preincubation group (group D) than in the enzyme-added 0 °C preincubation group (group C) (fig. 4).

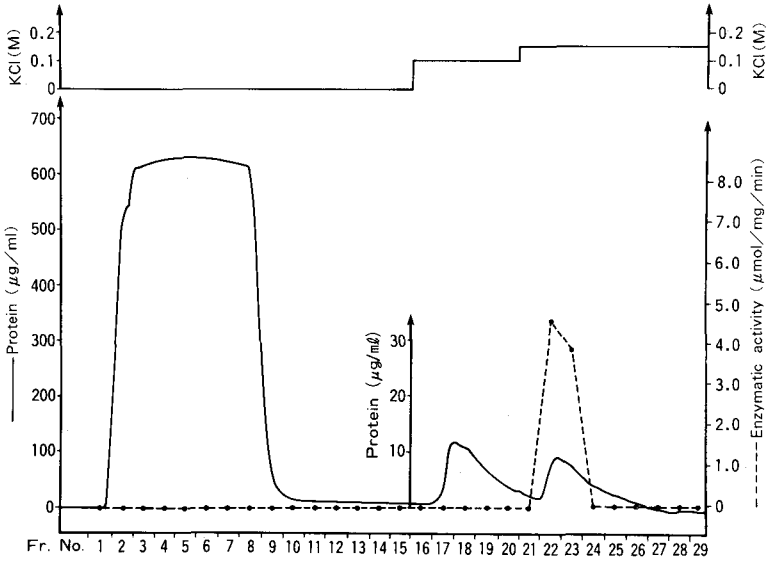

Figure 1. Elution profile of α-ketoaldehyde dehydrogenase activity after affinity column chromatography. A dotted line indicates the elution profile of enzyme activity. A solid line indicates the elution profile of proteins.

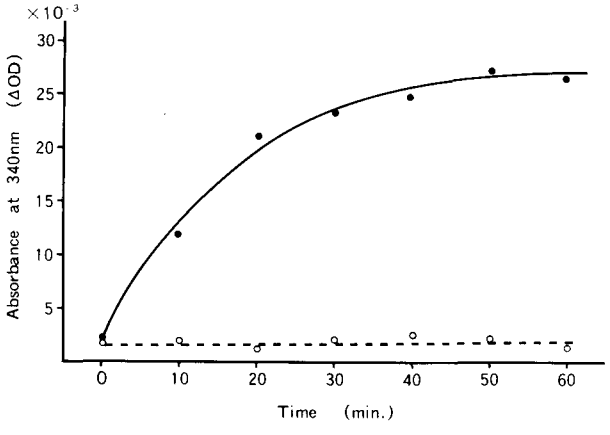

Figure 2. Changes of the enzymatic activity using an enzyme extract incubated with or without 1 mM p-CMB during 60 min at room temperature. A straight line indicates the reaction without p-CMB. A dotted line indicates the reaction with p-CMB. Results are means of 2 experiments.

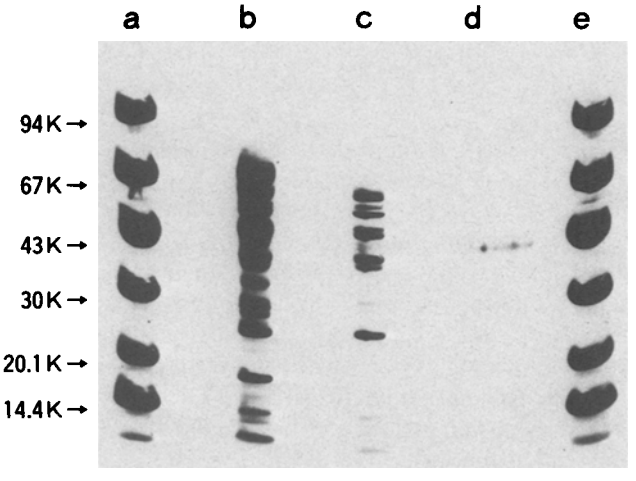

Figure 3. Gel after SDS-gradient PAGE stained with Coomassie blue.
a) Standard calibration kit proteins.
b) Crude enzyme solution extracted from acetone powder.
c) Enzyme solution passed through the DEAE-cellulose column.
d) Enzyme solution eluted with 0.15 M KCl from the affinity column.
e) Standard calibration kit proteins.

Discussion. Alfa-ketoaldehyde dehydrogenase is known to be present in the liver of the sheep and the goat[12,14]. However, it has not been reported that this enzyme is present in the human liver. We were able to extract α-ketoaldehyde dehydrogenase from the human liver by the method of Ray et al.[12] and to investigate its properties. α-Ketoaldehyde dehydrogenase reacts with methylglyoxal which is a dicarbonyl compound, and also with 3-DG as substrate. Pretreatment of α-ketoaldehyde dehydrogenase with p-CMB resulted in total loss of the enzymatic activity[12], suggesting the presence of a thiol group in the active site of the enzyme. The presence of a single band after SDS-PAGE suggests that the extracted α-ketoaldehyde dehydrogenase is a monomer.

The accumulation of advanced products of the Maillard reaction in long-lived proteins[15], such as collagen, nerve myelin and lens crystallin, has been given attention in relation to the etiology of diabetic complications and age-related pathologic states[4]. 2-(2-Furoyl)-4(5)-(2-furanyl)-1H-imidazole (FFI) has already been identified as an advanced glycosylation end-product (AGE)[4]. Attention has been focused not only on the production and accumulation of AGEs but also on the mechanism of their elimination. It has been reported that the Amadori compound fructose-lysine is converted in the presence of O_2 to carboxymethyllysine and erythronic acid and is then eliminated in the urine[16]. Vlassara et al.[17] have reported that there are receptors specific for FFI and

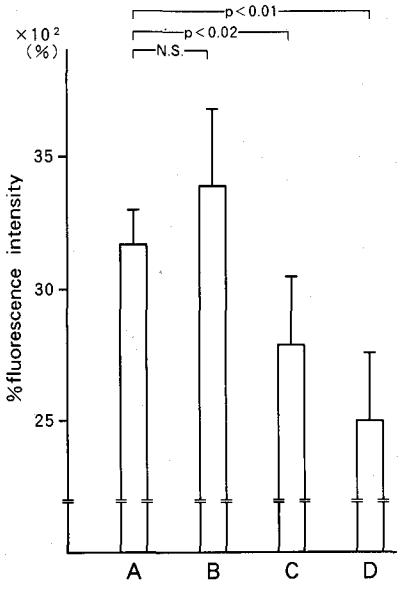

Figure 4. Fluorescence intensity of lysozyme incubated during 14 days after 2-h preincubation with or without α-ketoaldehyde dehydrogenase. Fluorescence intensity is expressed as percent increase of fluorescence compared to that of the corresponding sample stored at −40 °C after the same preincubation. A, B, C and D represent groups A, B, C and D as described in 'Materials and methods'. The data are the means ± SD of 4 experiments.

other AGEs in macrophages, and that there is a mechanism for the elimination of AGEs by macrophages. Brownlee et al.[18] have suggested that the aminoguanidine might inhibit the production of AGEs. We have assumed that α-ketoaldehyde dehydrogenase plays a role in the regulation of the accumulation of products of the Maillard reaction in the body. α-Ketoaldehyde dehydrogenase has been shown to act on carbonyl compounds, such as methylglyoxal and 3-DG. 3-DG is produced as an intermediate in the Maillard reaction and may act as a potent cross-linking agent[9, 10].

The results of the present study suggested that α-ketoaldehyde dehydrogenase acted on 3-DG and inhibited a 3-DG-stimulated increase in the fluorescence of lysozyme. It is thus possible that α-ketoaldehyde dehydrogenase might play a role in controlling pathogenesis attributed to aging, such as arteriosclerosis, both by preventing the progression of the Maillard reaction, and by inhibiting the formation of cross-links; both these reactions involve 3-DG.

Abbreviations: 3-deoxyglucosone: 3-DG; sodium dodecyl sulfate: SDS; diethyl-aminoethanol: DEAE; advanced glycosylation end-product: AGE; para-chloromercuribenzoate: *p*-CMB; 2-(2-furoyl)-4(5)-(2-furanyl)-1H-imidazole: FFI.

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0014-4754/89/050463-04$1.50 + 0.20/0

Solubilization and characterization of a ouabain-sensitive protein from transverse tubule membrane-junctional sarcoplasmic reticulum complexes (TTM-JSR) in cat cardiac muscle

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Received 1 March 1988; accepted 23 November 1988

Summary. A new glycoprotein of 31,500 dalton, which has a high affinity for ouabain, and is independent of (Na^+-K^+)-ATPase, was solubilized from transverse tubule membrane and junctional sarcoplasmic reticulum complexes (TTM-JSR) of cat cardiac muscle. This protein could be extracted only in small amounts from sarcolemma-plasma membrane (SLM-PL) fragments, suggesting that it indeed originates from the TTM-JSR.
Key words. Ouabain-sensitive protein; solubilization; characterization; TTM-JSR; cat heart.