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Stimulatory Action of Sodium Dodecyl Sulfate (SDS) on the Lecithin–Cholesterol Acyltransferase Reaction in Human Plasma

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The esterification of cholesterol in sonicated dispersions with lecithin–cholesterol molar ratios of 1.5 and 3.7 by human plasma was specifically stimulated by the addition of 5×10^{-4} M sodium dodecyl sulfate (SDS) but not by the addition of cetyltrimethylammonium chloride (CTAC) or Tween-20.

On the other hand, when residual protein fraction ($d > 1.210$ g/cm^3) of human plasma instead of human plasma was used as the enzyme source, the esterification of cholesterol in the dispersion with a molar ratio of 1.5 was also stimulated at 5×10^{-4} M SDS while that in the dispersion with a molar ratio of 3.7 was not stimulated. However, upon addition of high density lipoprotein (HDL) ($1.063 < d < 1.210$ g/cm^3), the esterification of cholesterol in the dispersion with a molar ratio of 3.7 (3.9) as well as that with a ratio of 1.5 (1.1) was also stimulated. Similarly, upon addition of 1×10^{-3} M mercaptoethanol, the stimulatory action of SDS on the esterification of cholesterol in the dispersion with a molar ratio of 3.7 (3.9) was observed even in the absence of exogenous HDL.

The stimulatory action of SDS on the acyltransferase reaction in human plasma is discussed on the basis of the above results.

Keywords——lecithin–cholesterol acyltransferase; human plasma; cholesterol; mercaptoethanol; sodium dodecyl sulfate

Human plasma contains lecithin–cholesterol acyltransferase (LCAT) [EC 2.3.1.43], which catalyzes the formation of cholesterol ester from lecithin and cholesterol.[1] We have previously reported that the esterification of cholesterol in a sonicated dispersion of lecithin and cholesterol mixture by human plasma is stimulated by the addition of sodium dodecyl sulfate (SDS) and also that the stimulatory action of SDS on cholesterol esterification is reversed by the addition of Ca^{2+} ion or bovine serum albumin.[2] However, the mechanism of the stimulatory action of SDS on cholesterol esterification by human plasma is not clear yet.

In this paper, we deal with the stimulatory action of SDS on cholesterol esterification by residual protein fraction ($d > 1.210$ g/cm^3) of human plasma in the presence or absence of exogenous high density lipoprotein (HDL) and mercaptoethanol.

Experimental

Materials——[7-^{3}H(N)]-Cholesterol was purchased from New England Nuclear Corp., (Boston, Mass., U.S.A.) and purified as described previously.[3] Lecithin was prepared from egg yolk by the method of Faure[4] and purified by silicic acid column chromatography. 5α-Cholestane was purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). The other chemicals used here were of reagent grade, purchased from Kanto Chemical Co. (Tokyo, Japan).

Preparation of Enzyme and Lipoprotein Fractions——As the source of LCAT, human plasma, residual protein fraction ($d > 1.210$ g/cm^3) of human plasma and $d = 1.210$—1.250 g/cm^3 fraction of human plasma were prepared as follows. Human plasma was obtained from outdated human blood containing 0.15 volume of anticoagulant solution (citric acid, sodium citrate, and glucose) by centrifugation at $1000 \times g$ for 15 min. The residual protein fraction was obtained by the same procedure as described previously[3] by ultracentrifugation of outdated human plasma. The $d =$

1.210—1.250 g/cm^3 fraction was prepared by the same procedure as described by Verdery[5] by ultracentrifugation of human plasma. This fraction was frozen at −70 °C and stored until used. The acyltransferase activity in this fraction was approximately 33 times purified with respect to plasma activity. HDL was separated from human plasma by the ultracentrifugal method described previously.[3] Human plasma, the residual protein fraction, the $d=1.210$—1.250 g/cm^3 fraction and HDL obtained here were dialyzed against Tris-HCl buffer, pH 7.0, ionic strength 0.1. The resulting human plasma and residual protein fraction were diluted with Tris-HCl buffer to give a protein content of 60—65 mg/ml. The protein content was determined by the procedure described by Lowry *et al.*[6] using crystalline bovine serum albumin as a standard.

Preparation of Substrate Dispersion——A sonicated dispersion of lecithin and ^{3}H-cholesterol mixture was prepared as described previously.[7] The contents of lipid phosphorus and cholesterol in the dispersion were determined by the procedure described by Muesing and Nishida.[8]

Enzyme Assay——The incubation mixture contained 0.1 ml of lecithin–^{3}H-cholesterol dispersion as a substrate and 0.2 ml of human plasma (65 mg protein/ml) or residual protein fraction (65 mg protein/ml) of human plasma. The final volume was ajusted to 0.5 ml with Tris-HCl buffer, pH 7.0, ionic strength 0.1. The various materials added to the incubation medium are described elsewhere in the text. The samples were placed in 15 ml screwcapped tubes, which were then flushed with N_2, sealed and incubated at 37 °C for 3 h with mechanical shaking. After incubation, lipids were extracted with 10 ml of chloroform–methanol (2 : 1, v/v) according to the procedure of Folch *et al.*[9] The chloroform extract was evaporated to dryness and the lipids obtained were separated by thin-layer chromatography on silica gel G plates, using *n*-hexane–diethylether–acetic acid (70 : 30 : 1, v/v) as the developing solvent. The lipids were located by staining with iodine vaper. The areas corresponding to cholesterol and cholesterol ester were each scraped off into a counting vial. The radioactivity was measured in a toluene (10 ml)–2,5-diphenyloxazole (PPO)–1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP) scintillation mixture using an Aloka liquid scintillation spectrometer (model LSC-502) as described previously.[10]

Determination of Plasma Cholesterol by Gas-Liquid Chromatography (GLC)——For each determination of free cholesterol, the reaction mixture containing 0.2 ml of human plasma (60 mg protein/ml), 0.2 ml of the $d=1.210$—1.250 g/cm^3 fraction (15 mg protein/ml) and 0.1 ml of Tris-HCl buffer (pH 7.4, ionic strength 0.1) was incubated at 37 °C for 3 h with mechanical shaking. After the incubation, the reaction was stopped by the addition of chloroform–methanol (2 : 1, v/v, 10 ml), then 5α-cholestane (25 μg) was added as an internal standard and the lipids were extracted as mentioned above. The concentration of free cholesterol, as its trimethylsilyl ether derivative, was determined by GLC as described by Marcel and Vezina.[11]

Results and Discussion

We first investigated whether the esterification of cholesterol in a sonicated dispersion of lecithin–cholesterol mixture by human plasma is stimulated by the addition of cetyltrimethylammonium chloride (CTAC) and Tween-20 as well as SDS. As shown in Fig. 1, the esterification of cholesterol was stimulated by 5×10^{-4} M SDS (curve 1) as reported previously by us,[2] but was not stimulated by CTAC (curve 2) and Tween-20 (curve 3) at the concentrations tested here. Thus, the esterification of cholesterol in a lecithin–cholesterol dispersion by human plasma was specifically stimulated by SDS, which is an anionic detergent, among the detergents tested here. The concentration of SDS required for the stimulation of the acyltransferase activity was lower than its critical micelle concentration (cmc, 1.3×10^{-3} M).

We next investigated whether the stimulatory action of SDS on cholesterol esterification is affected by the amount of lecithin, which has a solubilizing effect on cholesterol, in the substrate dispersion. As shown in Table I, the esterification of cholesterol by human plasma in the dispersions with lecithin/cholesterol molar ratios of 0.8—1.5 and 3.1—3.8 was stimulated at 5×10^{-4} M SDS. The stimulatory action of SDS on cholesterol esterification was slightly higher for the dispersions with molar ratios of 0.8—1.5 than for those with ratios of 3.1—3.8. The order of stimulatory potency of SDS on the esterification of cholesterol in the dispersions with molar ratios of 0.8—1.5 and 3.1—3.8 was unchanged even though the extent of cholesterol esterification varied with different plasma samples. The alteration of the acyltransferase activity in different plasma samples may be mainly due to the amounts of LCAT or lipoproteins. In addition, a similar result was previously found by us,[2] *i.e.*, the stimulatory effect of SDS on the acyltransferase activity in human plasma is higher for the dispersion with

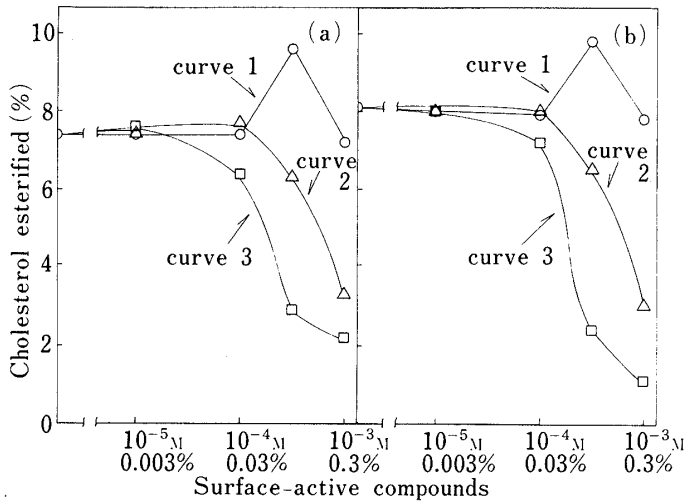

Fig. 1. Effect of Some Surface-Active Compounds on LCAT in Human Plasma

The incubation mixture contained 0.1 ml of a sonicated dispersion with a lecithin/cholesterol molar ratio of 3.1, 0.2 ml of human plasma and various amounts of surface-active compounds. The final volume was adjusted to 0.5 ml with Tris-HCl buffer, pH 7.4, ionic strength 0.1. Incubation was carried out at 37 °C for 3 h with constant shaking. The amounts of radioactivity and free cholesterol added to the incubation medium as sonicated dispersion were 1 μCi/0.50 μmol/ml of the dispersion. The concentration of Tween-20 in the incubation medium is presented as a percentage. The data represent the results obtained in two separate experiments (a and b).

Curve 1, SDS; curve 2, CTAC; curve 3, Tween-20.

TABLE I. Relationship between the Acyltransferase Activity and the Amount of Lecithin Added to the Incubation Medium in the Presence or Absence of SDS

Addition	Experiment I		Experiment II		Experiment III	
	Lecithin/cholesterol molar ratios in substrate disperisons					
	0.8	3.8	1.5	3.7	1.4	3.1
None	3.5 (100)	4.9 (100)	4.1 (100)	5.2 (100)	4.9 (100)	8.1 (100)
1×10^{-5} M SDS	3.5 (100)	4.9 (100)	4.1 (100)	5.2 (100)	4.9 (100)	8.0 (99)
1×10^{-4} M SDS	3.5 (100)	4.9 (100)	4.1 (100)	5.2 (100)	4.8 (98)	7.9 (98)
5×10^{-4} M SDS	4.5 (129)	5.8 (118)	5.2 (127)	6.3 (121)	6.4 (131)	9.8 (121)
1×10^{-3} M SDS	3.6 (103)	5.1 (104)	4.2 (105)	4.8 (92)	4.6 (94)	7.8 (96)

The incubation conditions were the same as in Fig. 1 except for the use of dispersion with molar ratios of 0.8, 3.8, 1.5, 3.7, 1.4 and 3.1. The amounts of radioactivity and free cholesterol in one ml of the dispersions with molar ratios of 0.8, 3.8, 1.5, 3.7, 1.4 and 3.1 were 1 μCi/0.40 μmol, 1 μCi/0.53 μmol, 1 μCi/0.43 μmol, 1 μCi/0.53 μmol, 1 μCi/0.42 μmol and 1 μCi/0.50 μmol, respectively. The values in parentheses are percentages of the values obtained in the absence of SDS (taken as 100%).

a molar ratio of 2.2 (179% of the value obtained in the absence of SDS) than for that with a molar ratio of 5.4 (143% of the value obtained in the absence of SDS). Accordingly, one of the stimulatory actions of SDS on the acyltransferase activity may be related to an increase of solubilization of cholesterol in the substrate dispersion. However, as shown in Table II, when endogenous free cholesterol in human plasma with partially purified LCAT ($d=1.210$—1.250 g/cm^3 fraction) before and after incubation was determined by GLC, the esterification of free cholesterol in human plasma was also slightly stimulated by the addition of 5×10^{-4} M SDS. Lacko *et al.*[12] have reported that molecular weight determinations of highly purified LCAT preparations on a calibrated gel column give a value of 95000 in the absence of SDS and about 50000 in the presence of SDS, indicating a monomer–dimer relationship. In

TABLE II. Determination of Cholesterol Esterification in Human Plasma by Gas-Liquid Chromatography

Addition	Lecithin–cholesterol acyltransferase activity (nmol/15 mg protein/3 h)		
	Experiment I	Experiment II	Experiment III
None	31.0 (100)	28.9 (100)	36.7 (100)
1×10^{-4} M SDS	28.9 (93)	28.7 (99)	35.1 (96)
5×10^{-4} M SDS	36.4 (117)	31.8 (110)	45.0 (123)
1×10^{-3} M SDS	30.0 (97)	16.3 (56)	27.1 (74)

The incubation mixture contained 0.2 ml of human plasma (12 mg protein), 0.2 ml of partially purified LCAT ($d=1.210$—$1.250\,g/cm^3$ fraction, 3 mg protein) and 0.1 ml of Tris-HCl buffer (pH 7.4). The incubation was carried out at 37 °C for 3 h. After incubation, the reaction was stopped by the addition of $CHCl_3$–MeOH (2 : 1 v/v, 10 ml), then 5α-cholestane (25 μg) was added as an internal standard and the lipids were extracted. The concentration of free cholesterol in the extracted lipids, as its trimethylsilyl derivative, was then determined GLC. The free cholesterol contents in the incubation mixtures of Experiments I, II and III before incubation were 64.9 μg/15 mg protein, 72.7 μg/15 mg protein and 78.2 μg/15 mg protein, respectively. The values in parentheses are percentages of the values obtained in the absence of SDS (taken as 100%).

addition, Doi and Nishida have recently reported that the transferase activity of LCAT increases after partial or complete desialylation by neuraminidase.[13] At present, it is not clear whether the release of sialic acid from LCAT is stimulated by the addition of SDS. In any event, the findings that the acyltransferase activity in human plasma was specifically stimulated by the addition of SDS among SDS, CTAC and Tween-20, and that the esterification of endogenous cholesterol in human plasma was also stimulated by the addition of SDS even though cholesterol was not added to the incubation medium as lecithin–cholesterol dispersion, suggest that the stimulatory action of SDS on the acyltransferase activity may be partly related to structural changes of HDL or LCAT rather than to alteration of the physical state of the substrate dispersion.

We have previously reported that, under experimental conditions similar to those used here, the maximal level of cholesterol esterification by residual protein fraction of human plasma is obtained at a lecithin/cholesterol molar ratio of about 3, and that upon increase or decrease in the molar ratio, cholesterol esterification is considerably reduced.[3] At the same time, we reported that the optimal concentration of HDL_3 ($1.125 < d < 1.210\,g/cm^3$) for the maximal acyltransferase activity is proportional to the amount of lecithin added to the incubation medium as substrate dispersion; the weight ratio of lecithin to HDL_3 protein for the maximal acyltransferase activity is 3.7—4.2.[3] Accordingly, the stimulatory action of SDS on the cholesterol esterification by human plasma may also be related to the amount of HDL in human plasma added to the incubation medium. Therefore, we investigated the effect of SDS on cholesterol esterification by residual protein fraction of human plasma in the presence or absence of exogenous HDL. As shown in Fig. 2, in the absence of exogenous HDL, the esterification of cholesterol in the dispersion with a molar ratio of 1.5 (curve 1) was stimulated at a concentration of 5×10^{-4} M SDS while that in the dispersion with a molar ratio of 3.7 (curve 2) was not stimulated. On the other hand, upon addition of 100 μg HDL per ml of the incubation medium, the stimulatory action of SDS was observed for the dispersion with a molar ratio of 3.7 (curve 4) as well as that with a ratio of 1.5 (curve 3). A similar result was obtained by the addition of Apo-HDL instead of HDL.

We have also reported that the acyltransferase activity in human plasma is stimulated by the addition of sulfhydryl agents such as mercaptoethanol, dithiothreitol, cysteine, thioglucose, and dimercaptosuccinate.[10,14] Furthermore, Verdery has also reported that mercapto-

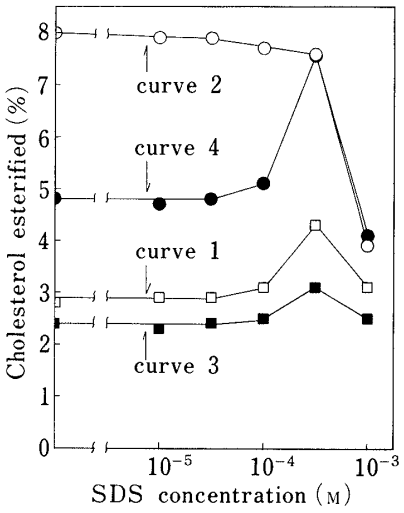

Fig. 2. Effect of SDS on the Acyltransferase Activity in Residual Protein Fraction of Human Plasma in the Presence or Absence of HDL.

The incubation conditions were the same as in Fig. 1 except for the use of residual protein fraction of human plasma instead of human plasma, and the addition of HDL. The amounts of radioactivity and free cholesterol in one ml of the dispersions with molar ratios of 1.5 and 3.7 were 1 μCi/0.43 μmol and 1 μCi/0.53 μmol, respectively. The data represent a typical set of reproducible results obtained in several in independent experiments.

Curve 1, a dispersion with a molar ratio of 1.5; curve 2, a dispersion with a molar ratio of 3.7; curve 3, a dispersion with a molar ratio of 1.5+100 μg HDL/ml of the incubation medium; curve 4, a dispersion with a molar ratio of 3.7+100 μg HDL/ml of the incubation medium.

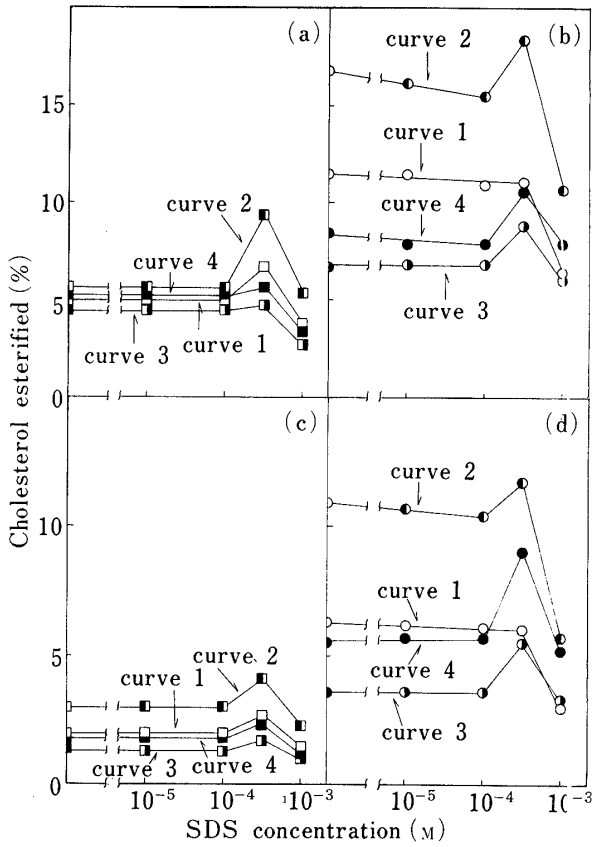

Fig. 3. Effects of Mercaptoethanol on the Acyltransferase Activity in Residual Protein Fraction of Human Plasma in the Presence or Absence of SDS and HDL

The incubation conditions were the same as in Fig. 1 except for the use of residual protein fraction of human plasma instead of human plasma, and the addition of HDL (100 μg/ml of the incubation medium) and 1×10^{-3} M mercaptoethanol. The amounts of radioactivity and free cholesterol in one ml of the dispersions with molar ratios of 1.5 (Fig. 3a), 3.7 (Fig. 3b), 1.1 (Fig. 3c) and 3.9 (Fig. 3d) used here were 1 μCi/0.47 μmol, 1 μCi/0.71 μmol, 1 μCi/0.45 μmol and 1 μCi/0.69 μmol, respectively. The data were obtained two separate experiments (Fig. 3a, b and Fig. 3c, d).

Curve 1, none; curve 2, mercaptoethanol; curve 3, HDL; curve 4, mercaptoethanol+HDL.

ethanol and dithiothreitol stimulate the acyltransferase activity while cysteine and reduced glutathione inhibit it.[5] At the same time, he suggested that, in view of the contrasting effects of different sulfhydryl agents on the acyltransferase activity, the role of sulfhydryl groups in the acyltransferase reaction is complex.[5] Therefore, the stimulatory action of SDS on the acyltransferase activity in residual protein fraction of human plasma was investigated in the presence or absence of mercaptoethanol. As shown in Fig. 3, the acyltransferase activity was remarkably stimulated by the addition of 1×10^{-3} M mercaptoethanol. The stimulatory effect of mercaptoethanol was greater for the dispersions with molar ratios of 3.7 (Fig. 3b) and 3.9 (Fig. 3d) than for those with ratios of 1.5 (Fig. 3a) and 1.1 (Fig. 3c), respectively. In particular, the stimulatory action of SDS on the esterification of cholesterol in the dispersions with molar ratios of 3.7 and 3.9 (curve 1 in Fig. 3b, d) was not observed in the absence of exogenous HDL. However, upon addition of 1×10^{-3} M mercaptoethanol, the stimulatory action of SDS

on the esterification of cholesterol was observed as an additive action of SDS and mercaptoethanol (curve 2 in Fig. 3b, d). Similarly, the stimulatory action of SDS on the esterification of cholesterol in the dispersions with molar ratios of 1.5 and 1.1 in the absence of exogenous HDL was also further increased by the addition of mercaptoethanol (curve 2 in Fig. 3a, c). On the other hand, the extent of stimulatory action of SDS on the esterification of cholesterol in the dispersions with molar ratios of 1.5 and 1.1 (curve 4 in Fig. 3a, c) in the presence of exogenous HDL and mercaptoethanol was little increased as compared with those in the presence of exogenous HDL alone (curve 3).

It has been suggested that the stimulatory effect of mercaptoethanol on the acyltransferase reaction is due to the protection of sulfhydryl groups in the acyltransferase by its reducing action[15] and also that the disulfide linkages in the acyltransferase are necessary for the acyltransferase activity.[5] In any event, the stimulatory action of mercaptoethanol on the acyltransferase reaction in residual protein fraction of human plasma observed here may also be mainly due to the reduction of an oxidized form of the acyltransferase. However, the stimulatory action of mercaptoethanol on the acyltransferase reaction may be partially related to the transformation of endogenous HDL to a more active form for the acyltransferase reaction. Experiments to clarify this problem are in progress.

References and Notes

1) J. A. Glomset, *J. Lipid Res.*, **9**, 155 (1968).
2) M. Nakagawa and S. Kojima, *J. Biochem.*, **80**, 729 (1976).
3) M. Nakagawa and T. Nishida, *Biochim. Biophys. Acta*, **296**, 577 (1973).
4) M. Faure, *Bull. Soc. Chim. Biol.*, **32**, 503 (1950).
5) R. B. Verdery, *Biochem. Biophys. Res. Commun.*, **98**, 494 (1981).
6) O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
7) M. Nakagawa and M. Uchiyama, *Biochem. Pharmacol.*, **23**, 1641 (1974).
8) R. A. Muesing and T. Nishida, *Biochemistry*, **10**, 2952 (1971).
9) J. Folch, M. Less and G. H. Sloane-Stanley, *J. Biol. Chem.*, **226**, 497 (1957).
10)) M. Nakagawa, M. Takamura and S. Kojima, *J. Biochem.*, **81**, 1011 (1977).
11) Y. L. Marcel and C. Vezina, *Biochim. Biophys. Acta*, **306**, 497 (1973).
12) A. G. Lacko, K. G. Varma, H. L. Rutenberg and L. A. Soloff, *Scand. J. Clin. Lab. Invest.*, **33**, 29 (1974).
13) Y. Doi and T. Nishida, *J. Biol. Chem.*, **258**, 5840 (1983).
14) M. Nakagawa, S. Motojima, Y. Fujimoto, K. Furusawa, K. Murata and S. Kojima, *Chem. Pharm. Bull.*, **30**, 1884 (1982).
15) A. K. Soutar, C. W. Graner, H. N. Baker, J. T. Sparrow, R. L. Jackson, A. M. Gotto and L. C. Smith, *Biochemistry*, **14**, 3057 (1975).