Vol. 80, No. 3, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 14, 1978 Pages 623-630

THE AMINO ACID SEQUENCE OF HUMAN APOA-I, AN APOLIPOPROTEIN ISOLATED FROM HIGH DENSITY LIPOPROTEINS

H.B. Brewer, Jr., T. Fairwell, A. LaRue,
R. Ronan, A. Houser, and T.J. Bronzert

Molecular Disease Branch, National Heart, Lung, and Blood Institute,
National Institutes of Health, Bethesda, MD 20014, U.S.A.

Received December 23,1977

Summary: The complete amino acid sequence of human A-I has been determined by manual and automated Edman degradation of intact and peptide fragments of A-I. A-I is a single chain protein of 243 residues with the following amino acid composition: Asp_{16}, Asn_5, Thr_{10}, Ser_{15}, Glu_{27}, Gln_{19}, Pro_{10}, Gly_{10}, Ala_{19}, Val_{13}, Met_3, Leu_{37}, Tyr_7, Phe_6, Trp_4, Lys_{21}, His_5, and Arg_{16}. The amino acid sequence contains no linear segments of hydrophobic or hydrophilic residues. A detailed correlation of the amino acid sequence, conformation, and self association of A-I will add further insight into the molecular mechanisms involved in protein-protein and protein-lipid interactions.

Introduction

High density lipoproteins (HDL) are a polydisperse collection of plasma lipoproteins containing a number of different apolipoproteins (1-3). Two apolipoproteins, A-I and A-II, constitute greater than 90% of the protein moiety of human HDL. The physical properties of human A-I have been described and A-I has been shown to self-associate with an increase in α-helical structure concomitant with association (4-7). Analysis of the primary structure of several of the human apolipoproteins has identified segments of the sequences which could be modeled into amphipathic helices (8-10). These helices have been proposed to be of importance in lipid-protein interaction. The complete 245 residue sequence of human A-I has been reported and the areas of amphipathic helix identified (11-13). Structural analysis of human A-I in our laboratory is at variance with the published sequence. The present report summarizes the results of our investigation of the sequence of human A-I. A detailed description of the primary structure of A-I will be reported elsewhere (LaRue, A., Ronan, R., Houser, A., Fairwell, T., and Brewer, H.B. Jr.; Fairwell, T.,

0006-291X/78/0803-0623$01.00/0

Ronan, R., LaRue, A., Bronzert, T., Houser, A., and Brewer, H.B. Jr., to be published).

MATERIALS AND METHODS

Purification of A-I HDL obtained from normal volunteers, was isolated by preparative ultracentrifugation between salt densities 1.063 and 1.21 g/ml (14), and delipidated with chloroform-methanol (2:1, v/v) (15). A-I was isolated from delipidated HDL by gel filtration on Sephadex G-200 (superfine) in 8M urea.

Chemical and Enzymatic Cleavage of A-I Cleavage of A-I into peptide fragments was performed by the following techniques: cyanogen bromide [48 hr., 500:1 (molar ratio), 25°C], trypsin [0.75-1.5 hr., 1:20 to 1:50 (wt/wt), 37°C], citraconylation followed by trypsin [5 hr., 1:25 (wt/wt), 37°C], hydroxylamine [3 hr., 2M hydroxylamine-HCl, pH 10.3, 45°C], BNPS-skatole, [skatole 2-(2-nitrophenylsulfenyl)-3-methylindole, 16] [24 hr., 20:1 (molar ratio), 37°C], cyclohexandione [2.5 hr., 40.1 (molar ratio) 25°C], and Staphylococcus aureus protease [24 hr., 1:50 (wt/wt), 37°C].

Isolation of A-I Peptides Peptides were purified to homogeneity by gel permeation and ion exchange chromatography (see results section). The isolated peptides were assayed for purity by techniques detailed in previous reports (15, 17-18), and included amino acid analysis, thin layer chromatography, disc gel electrophoresis, and Edman amino-terminal analysis.

Phenylisothiocyanate Degradations A-I and peptide fragments were sequenced by manual or automated Edman-phenylisothiocyanate degradations (18). Automated degradations were performed on the Beckman Sequencer employing a 1M Quadrol buffer system. The phenylthiohydantoin amino acids (PTH amino acids) were identified by gas-liquid chromatography, (2/3 CFC Blend, 19), high pressure-liquid chromatography (Zorbax ODS, 20), and mass spectroscopy (Finnegan Quadrapole mass spectrometer, 21, 22). PTH amino acids were quantitated by gas-liquid and high pressure-liquid chromatography.

RESULTS

A-I isolated for sequence analysis was a single band by electrophoresis in sodium dodecyl sulfate, and a single amino-terminal amino acid, aspartic acid, was obtained with the Edman procedure. A-I is a single chain protein composed of 243 amino acids. The complete amino acid composition, determined by timed acid hydrolysis (Table I), peptide composition, and Edman degradation is as follows: Asp_{16}, Asn_5, Thr_{10}, Ser_{15}, Glu_{27}, Gln_{19}, Pro_{10}, Gly_{10}, Ala_{19}, Val_{13}, Met_3, Leu_{37}, Tyr_7, Phe_6, Trp_4, Lys_{21}, His_5, and Arg_{16}.

The complete amino acid sequence of A-I, determined by Edman degradation of intact A-I and peptide fragments, is illustrated in Figure 1 (large arrows and small arrows denote automated and manual Edman degradation respectively). The sequence of the initial 53 residues was obtained by automated Edman de-

TABLE I

Amino Acid Analysis of Native, and Peptide Fragments of ApoA-I*

Amino Acid	ApoA-I†	CB-1	CB-2	CB-3	CB-4	A-I-T-1	A-I-T-2	A-I-T-3	CB-1-CT-3	CB-1-CT-4	CB-2-T-5	CB-4-CT-2-SP-3
Aspartic Acid	20.53 (21)	11.82 (12)	3.05 (3)	–	5.99 (6)	–	–	–	5.00 (5)	2.03 (2)	–	1.04 (1)
Threonine	9.64 (10)	4.84 (5)	–	–	4.93 (5)	–	–	–	1.89 (2)	1.95 (2)	–	2.10 (2)
Serine	14.72 (15)	6.97 (7)	.93 (1)	1.06 (1)	5.88 (6)	.89 (1)	–	.98 (1)	4.78 (5)	–	–	–
Glutamic Acid	45.71 (46)	14.75 (14)	6.82 (7)	12.36 (12)	13.47 (13)	2.05 (2)	4.27 (4)	2.24 (2)	3.05 (3)	9.24 (9)	3.13 (3)	.98 (1)
Proline	10.43 (10)	4.06 (4)	1.10 (1)	1.89 (2)	3.05 (3)	–	–	.95 (1)	–	.99 (1)	–	–
Glycine	10.23 (10)	5.17 (5)	–	2.03 (2)	3.30 (3)	–	–	1.04 (1)	2.05 (2)	1.98 (2)	–	–
Alanine	18.99 (19)	1.98 (2)	1.00 (1)	1.90 (2)	13.85 (14)	–	–	–	1.05 (1)	–	–	–
Valine	13.32 (13)	6.62 (7)	2.09 (2)	1.00 (1)	2.88 (3)	–	–	–	2.08 (2)	1.07 (1)	–	–
Methionine	3.00 (3)	– (1)†	– (1)†	– (1)†	–	.69 (1)	.92 (1)	.86 (1)	–	– (1)†	– (1)†	–
Leucine	37.42 (37)	11.06 (11)	2.00 (2)	7.22 (7)	17.99 (17)	–	1.06 (1)	2.10 (2)	6.30 (6)	3.02 (3)	–	1.06 (1)
Tyrosine	7.03 (7)	1.78 (2)	.96 (1)	.91 (1)	3.06 (3)	–	.96 (1)	–	.90 (1)	–	–	.86 (1)
Phenylalanine	5.85 (6)	3.23 (3)	1.00 (1)	–	2.08 (2)	–	–	–	2.03 (2)	.96 (1)	–	–
Lysine	20.95 (21)	5.86 (6)	4.80 (5)	3.05 (3)	7.05 (7)	1.08 (1)	–	–	3.08 (3)	1.04 (1)	–	2.12 (2)
Histidine	4.50 (5)	–	–	1.03 (1)	3.91 (4)	–	–	–	–	–	–	–
Arginine	15.67 (16)	4.09 (4)	–	2.93 (3)	9.15 (9)	–	.98 (1)	1.00 (1)	.97 (1)	.96 (1)	–	–

* All values expressed as molar ratios of the constituent amino acids following 24 hr hydrolysis at 108°C. The numbers in parenthesis are the assumed numbers of residues per mol.

† Extrapolated values from 24, 48, 72 hr timed acid hydrolyses.

‡ Identified as homoserine - homoserine lactone.

gradation on intact A-I (Fig. 1).

Four major peptides were isolated following cyanogen bromide (CB) cleavage of A-I (Table I, Fig. 2). These peptides were designated CB-1 (86 residues), CB-2 (26 residues), CB-3 (36 residues), and CB-4 (95 residues). The sequence of each of the four CB peptides was obtained as follows:

CB-1 (Residues 1-86): Tryptic digestion of citraconylated CB-1 yielded four peptides (Fig. 1). Peptide CB-1-CT-1 was isolated on Bio-Gel P-30 (25% acetic acid), CB-1-CT-3 on Bio-Gel P-30 followed by DEAE cellulose chromatography (gradient, 0.01M-0.20M NH_4HCO_3, pH 7.95), and CB-1-CT-2 and CB-1-CT-4 by Bio-Gel P-30 and DEAE cellulose chromatography (gradient, 0.01M-.30M NH_4HCO_3, pH 8.0). Peptides CB-1-CT-1 and CB-1-CT-2 corresponded to residues 1-10, and 11-27 determined by automated degradation of A-I (Fig. 1). CB-1-CT-4 contained a single methionine (homoserine-homoserine lactone) residue, indicating that CB-1-CT-3 and CB-1-CT-4 corresponded to the third, and carboxyl-terminal peptides of CB-1 respectively (Fig. 1, Table 1). The sequence of the initial 26 residues of CB-1-CT-3 was determined by manual degradation (Fig. 1). The carboxyl-terminal sequence of CB-1-CT-3 was completed by cleavage at the single tryptophan

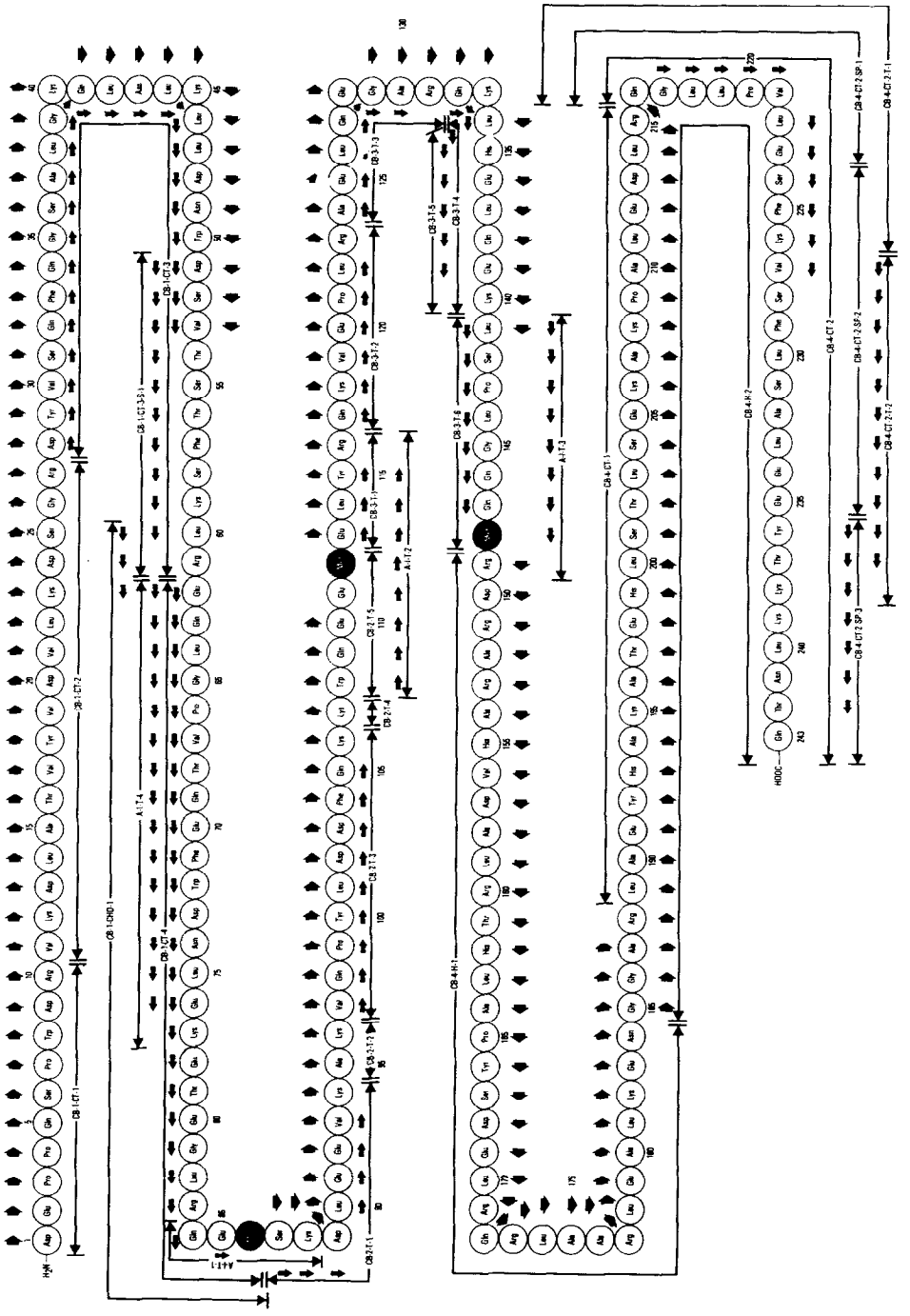

FIGURE 1. Amino acid sequence of ApoA-I

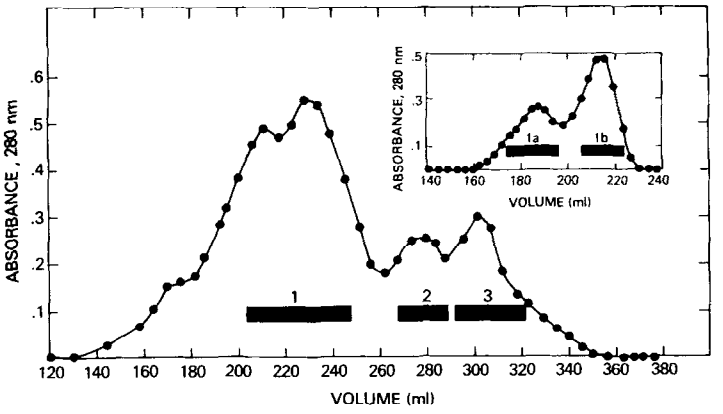

FIGURE 2. Bio-Gel P-10 chromatography (25% acetic acid) of the cyanogen bromide peptides of ApoA-I. Inset - Rechromatography of fraction 1 on Bio-Gel P-30. Each of the peptides was purified to homogeneity by chromatography on DEAE cellulose: Fraction 1a (CB-4) and 1b (CB-1)-gradient, 0.01-025M Tris-6M urea, pH 8.0; fraction 2 (CB-3) gradient, 0.01-0.17M NH_3HCO_3, pH 8.0; and fraction 3 (CB-2) gradient 0.01-0.18M NH_4HCO_3, pH 8.0.

(residue 50) with BNPS-skatole, isolation of the carboxyl-terminal peptide (CB-1-CT-3-S-1) by DEAE cellulose chromatography (gradient, 0.01-0.3 NH_4HCO_3, pH 8.0) and manual Edman degradation (residues 51-61, Fig. 1). The entire sequence of CB-1-CT-4 was completed by manual degradation (residues 62-86, Fig. 1). The carboxyl-terminal residue homoserine (lactone) was identified by amino acid analysis. The sequence of residues 62-77 was also determined by manual degradation of peptide A-I-T-4 obtained from tryptic digestion of intact A-I and isolation by DEAE cellulose chromatography (gradient, 0.01-0.17M NH_4HCO_3, pH 8.2) (Fig. 1). To overlap residues 61 and 62, CB-1 was modified with cyclohexandione, cleaved with trypsin, and the carboxyl-terminal peptide isolated by DEAE cellulose chromatography (gradient, 0.01-0.20M NH_4HCO_3, pH 8.0) (CB-1-CHD-1, residues 60-86, Fig. 1). Amino-terminal degradation of CB-1-CHD-1 revealed leucine, arginine, and glutamic acid thus completing the sequence of CB-1.

CB-2 (Residues 87-112): The sequence of 24 of the 26 residues of CB-2 was elucidated by automated Edman degradation of CB-2 modified with 4-sulfonated

phenylisothiocyanate [SPITC] (Fig. 1, residues 87-110). Tryptic cleavage of CB-2 yielded four peptides and lysine (Fig. 1). Peptides CB-2-T-1 and CB-2-T-3 were isolated by DEAE cellulose chromatrography (gradient, 0.01M-.13M, NH_4HCO_3, pH 8.0), and CB-2-T-4 and CB-2-T-5 by DEAE followed by CMC cellulose chromatography (.01M NH_4HCO_3, pH 8.1) [Table I]. Manual degradation of CB-2-T-1 and CB-2-T-3 confirmed the sequence of residues 87-94 and 97-106 respectively (Fig. 1). The sequence of the carboxyl two amino acids of CB-2 (residues 111-112) was obtained by the degradation of methionine overlap peptide, A-I-T-2, outlined below.

CB-3 (Residues 113-148): The initial 29 residues of CB-3 were determined by automated degradation of SPITC modified CB-3 (Fig. 1, residues 113-141). Tryptic digestion and chromatography on DEAE cellulose (gradient, 0.1-.15M NH_4HCO_3, pH 8.0) of CB-3 yielded 6 peptides (Fig. 1). Manual degradation of peptides CB-3-T-1 to CB-3-T-5 confirmed the sequence of residues 113-140 (Fig. 1). The remainder of the sequence of CB-3 (residues 141-148) was completed by manual degradation of the methionine overlap peptide, A-I-T-3 described below.

CB-4 (Residues 149-243): The initial 39 residues of CB-4 were sequenced by automated degradation (Fig. 1, residues 149-187). CB-4 was cleaved into two peptides (CB-4-H-1 and CB-4-H-2) at the Asn-Gly sequence (Fig. 1, residues 184-185) by hydroxylamine. CB-4-H-1 and CB-4-H-2 were isolated by gel permeation chromatography on Bio-Gel P-30 (25% acetic acid). CB-4-H-2 was automatically degraded for 32 cycles (Fig. 1, residues 185-216). These two automated degradations established the initial 67 residues of CB-4 (Fig. 1). The carboxyl-terminal peptide of CB-4 was obtained by tryptic digestion of citraconylated CB-4, and isolation of peptide CB-4-CT-2 by Bio-Gel P-30 (25% acetic acid) followed by DEAE cellulose chromatography (gradient, 0.01-0.10M NH_4HCO_3, pH 8.0), (Fig. 1, residues 216-243). The initial sequence of CB-4-CT-2 (Fig. 1, residues 216-227) was determined by manual degradation. Tryptic digestion and DEAE cellulose chromatography (gradient, 0.01-0.3M NH_4HCO_3, pH 8.0) of deblocked

CB-4-CT-2 yielded peptide, CB-4-CT-2-T-2, which was manually degraded overlapping residues 227 and 228, and completing the sequence of residues 227-238 (Fig. 1). The carboxyl-terminal sequence of CB-4 was determined on peptide CB-4-CT-2-SP-3 obtained by cleavage of CB-4-CT-2 with Staphylococcus aureus protease and chromatography on DEAE cellulose (gradient 0.01-0.2M NH_4HCO_3, pH 8.0) [Fig. 1, Table I]. Manual degradation of CB-4-CT-2-SP-3 overlapped residues 236-238, and completed the sequence of residues 238-243. The carboxyl-terminal residue, glutamine, was identified by amino acid analysis.

The alignment of CB peptides CB-1 to CB-4 was ascertained by tryptic digestion of A-I, and isolation of the three methionine containing peptides, A-I-T-1 (DEAE cellulose chromatography, gradient, 0.01-0.17M NH_4HCO_3, pH 8.2), A-I-T-2 (DEAE cellulose chromatography, Sephadex G-25 [.2M NH_4HCO_3], followed by DEAE cellulose elution (.01M NH_4HCO_3, pH 8.2), and A-I-T-3 (DEAE cellulose chromatography followed by Sephadex G-50 [.2M NH_4HCO_3]). Manual degradation of A-I-T-2 (residues 108-116) and A-I-T-3 (residues 141-149) completed the amino acid sequence of A-I (Fig. 1).

DISCUSSION

The sequence of human A-I described here is significantly different from the structure of human A-I previously reported (11-13). In our studies, A-I is 243 versus 245 amino acids in length, and 23 of the 243 residues are different including the insertion of amino acid residues aspartic acid-serine (residues 51-52), glutamic acid (residue 111), lysine (residue 239), and deletion of glutamine (residue 74 [11-13]), and tryptic peptide T-12 (residues 77-81 [11-13]) in the sequence. The reason for these differences in the primary structure of human A-I remains to be elucidated.

A prerequisite for our understanding of the molecular organization of plasma lipoproteins is a detailed understanding of the molecular properties of the individual protein constituents. A correlation of the amino acid sequence, conformation, and self-association of A-I and other plasma

apolipoproteins will add further insight into the molecular mechanisms involved in protein-protein and protein-lipid interactions.

REFERENCES

1. Alaupovic, P. (1972) In "Protides of the Biological Fluids" (H. Peeters, ed.) pp. 9-19, Permgamon Press, Oxford and New York.
2. McConathy, W.J., and Alaupovic, P. (1976) Biochem. 15, 515-520.
3. Osborne, J.C., Jr., and Brewer, H.B. Jr., (1977) Adv. Prot. Chem. 31, 253-337.
4. Gwynne, J., Brewer, H.B. Jr., and Edelhoch, H. (1974) J. Biol. Chem. 249, 2411-2416.
5. Stone, W.L., and Reynolds, J.A. (1975) J. Biol. Chem. 250, 8045-8048.
6. Vitello, L.B. and Scanu, A.M. (1976) J. Biol. Chem. 251, 1131-1136.
7. Formisano, S., Brewer, H.B. Jr., and Osborne, J.C. Jr., J. Biol. Chem. In press.
8. Assmann, G., and Brewer, H.B. Jr. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 1534-1538.
9. Segrest, J.P., Jackson, R.L., Morrisett, J.D., and Gotto, A.M. (1974) FEBS Lett. 38, 247-253.
10. Jackson, R.L., Morrisett, J.D., and Gotto, A.M. Jr. (1976) Physiol. Rev. 56, 259-316.
11. Baker, H.N., Delahunty, T., Gotto, A.M. Jr., and Jackson, R.L. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 3631-3634.
12. Delahunty, T., Baker, N. Gotto, A.M. Jr., and Jackson, R.L. (1975) J. Biol. Chem. 250, 2718-2724.
13. Baker, H.N., Gotto, A.M. Jr., and Jackson, A.M. (1975) J. Biol. Chem. 250, 2725-2738.
14. Havel, R.J., Eder, H.A., and Bragdon, J.H. (1955) J. Clin. Invest. 34, 1345-1353.
15. Lux, S.E., John, K.M., and Brewer, H.B. Jr. (1972) J. Biol. Chem. 247, 7510-7518.
16. Omenn, G.S., Fontana, A., and Anfinsen, C.B. (1970) J. Biol. Chem. 245, 1895-1902.
17. Lux, S.E., John, K.M., Ronan, R., and Brewer, H.B. Jr. (1972) J. Biol. Chem. 247, 7519-7527.
18. Brewer, H.B. Jr., Shulman, R., Herbert, P., Ronan, R., and Wehrley, K. (1974) J. Biol. Chem. 249, 4975-4984.
19. Pisano, J.J., Bronzert, T., Brewer, H.B. Jr. (1972) Anal. Biochem. 45, 43-59.
20. Zimmerman, C.L., Apella, E., and Pisano, J.J. (1976) Anal. Biochem. 77, 569-573.
21. Fales, H.M., Nagai, Y., Milne, G.W.A., Brewer, H.B. Jr., Bronzert, T.J. and Pisano, J.J. (1971) Anal. Biochem. 43, 288-299.
22. Fairwell, T., and Brewer H.B. Jr. (1973) Fed. Proc. 32, 2446.