## Assymetry in the Distribution of Basic Amino Acid Residues in the Moderately Lysine-Rich Histone F2b from Calf Thymus

Though the exact biological role of histones is unknown, it is assumed generally that these basic nuclear proteins function in the process of genetic restriction of DNA templates. Although the complete amino acid sequence of the arginine-rich histone F2al (or IV) from calf thymus and pea seedlings was reported recently[1], only rudimentary information is available on the structure of the lysine-rich histones F1 and F2b (ref. [2,3]).

Crude F2b histone prepared by a selective extraction of calf thymus chromatin[4,5] was chromatographed on carboxymethyl cellulose (BioRad Cellex CM, 0.71 meq/g) as was described previously[4]. The protein recovered by dialysis and lyophilization was purified by chromatography on sulfoethyl cellulose (BioRad Cellex SE, 0.23 meq/g) column $32 \times 360$ mm using a linear gradient from $0.1\,M$ HCOONa in $4\,M$ urea to $0.3\,M$ HCOONa and $0.3\,M$ HCOOH in $6\,M$ urea for elution. The main peak was rechromatographed on the same resin ($22 \times 500$ mm column, linear gradient from $0.1\,M$ HCOONa in $8\,M$ urea to $0.3\,M$ HCOONa and $0.3\,M$ HCOOH in $8\,M$ urea). Finally, the F2b histone was purified by gel filtration on Sephadex G-75 column ($24 \times 1800$ mm) using $2.5\,M$ guanidine hydrochloride in $0.01\,N$ HCl for elution. The F2b histone from calf thymus was homogeneous in 2 different systems of polyacrylamide electrophoresis[6,7] and its amino acid composition is shown in Table I. The COOH terminal amino acid of F2b histone is lysine; the $NH_2$ terminal amino acid is proline.

Digestion of F2b histone with trypsin (Worthington, TPCK treated) resulted in the formation of an insoluble precipitate (core), which was a pentadecapeptide (peptide T21), and in 19 soluble peptides contained in the digest. Free lysine and arginine were also present in this digest. The soluble peptides were isolated by chromatography on Dowex 50W resin at 50 °C using a sequence of 4 pyridine acetate buffers reported by GUIDOTTI et al.[8] followed by $0.05\,N$ NaOH. The amino acid sequences of individual tryptic (T) peptides determined by the Edman substractive procedure[9] are shown in the Figure.

Since the F2b histone contains 2 methionine residues, a 150 mg sample was cleaved with cyanogen bromide at room temperature for 20 h[10,11]. The dried fragments were fractionated by gel filtration on Sephadex G-50 ($24 \times 1800$ mm column and $0.01\,N$ HCl saturated with $CH_3Cl$ as eluent). Most of the material emerged close to the void volume followed by a small peak consisting of a tripeptide (GLY-ILE-Homoserine). This indicates that the 2 methionine residues are located close together in the center of the molecule.

The large fragments were separated by rechromatography of the leading peak resulting from the Sephadex G-50 filtration on a Sephadex G-75 column, employing similar conditions. A small peak of F2b histone was followed closely by a large, assymetrical peak containing 2 fragments of F2b histone. The fragments were isolated by gel filtration on Biogel P60 (conditions similar as for the Sephadex filtration) and their amino acid composition is shown in Table I. From the partial amino acid sequence of the tryptic 'core' (T21) can be concluded that the smaller and more basic fragment BCN I is the $NH_2$ terminal portion of F2b histone, and the larger and less basic fragment BCN II represents the COOH terminal portion of this protein. The number of amino acid residues in both fragments (58 and 65) also shows that the 2 methionone residues are situated approximately in the center of F2b histone.

To ascertain the distribution of basic amino acid residues, 500 ml of the F2b histone were maleylated[1]. The maleylated protein was digested by trypsin (Worthington, TPCK treated) and the peptides (TM peptides) were fractionated by chromatography on Dowex 50W resin at 45 °C and by gel filtration on Sephadex G-50 and G-25 columns. A total of 8 TM peptides was recovered. In addition to the 4 TM peptides (TM4, TM5, TM6, and TM7) which are identical to tryptic peptides T5, T10, T17, and T20, a small peptide LYS-SER-ARG (TM2) and 3 large peptides TM1, TM3, and TM8 were isolated and analyzed.

Table I. Amino acid composition of whole protein, tryptic core and cyanogen bromide fragments from calf thymus Fèb histone

| Amino acid | F2b histone | | Tryptic core | | Fragment BCNI | | Fragment BCNII | |
|---|---|---|---|---|---|---|---|---|
| | *M*% | R | *M*% | R | *M*% | R | *M*% | R |
| Lysine | 16.6 | 20 | 0.1 | – | 24.5 | 14 | 9.5 | 6 |
| Histidine | 2.2 | 3 | – | – | 1.8 | 1 | 3.1 | 2 |
| Arginine | 6.2 | 8 | 6.7 | 1 | 5.3 | 3 | 7.9 | 5 |
| Aspartic acid | 4.9 | 6 | 20.3 | 3 | 3.5 | 2 | 6.2 | 4 |
| Threonine | 6.1 | 8 | 0.1 | – | 3.4 | 2 | 9.3 | 6 |
| Serine | 10.7 | 14 | 6.5 | 1 | 12.1 | 7 | 10.3 | 7 |
| Glutamic acid | 7.6 | 10 | 6.6 | 1 | 7.2 | 4 | 9.6 | 6 |
| Proline | 4.8 | 6 | – | – | 8.8 | 5 | 1.6 | 1 |
| Glycine | 5.5 | 7 | 6.7 | 1 | 5.2 | 3 | 4.8 | 3 |
| Alanine | 10.1 | 13 | 6.9 | 1 | 10.6 | 6 | 10.4 | 7 |
| Valine | 7.0 | 9 | 6.8 | 1 | 8.7 | 5 | 6.2 | 4 |
| Methionine | 1.5 | 2 | 12.9 | 2 | – | – | – | – |
| Isoluecine | 4.9 | 6 | 13.6 | 2 | 1.8 | 1 | 6.4 | 4 |
| Leucine | 4.9 | 6 | 0.2 | – | 1.9 | 1 | 7.9 | 5 |
| Tyrosine | 3.8 | 5 | – | – | 5.1 | 3 | 2.9 | 2 |
| Phenylalanine | 1.6 | 2 | 13.4 | 2 | – | – | 3.2 | 2 |
| Homoserine | – | – | – | – | 1.6 | 1 | – | – |

Amino acids are expressed as mole percents of all amino acids recovered. All serine values were corrected (10%) for hydrolytic losses. *M*%, mole percent; R, number of residues.


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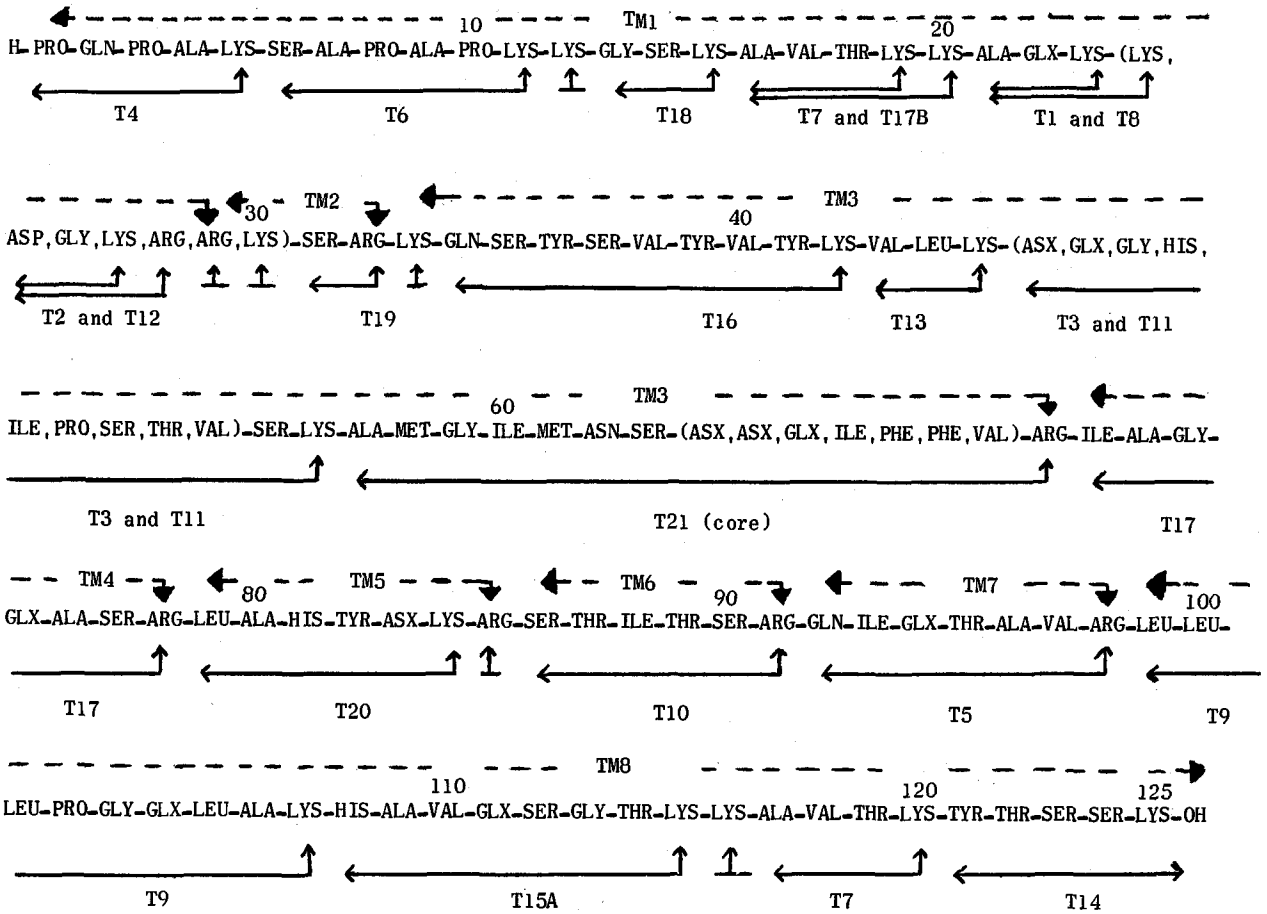


Composition of tryptic peptides and partial amino acid sequence of moderately lysine-rich histone F2b from calf thymus. Solid lines: tryptic peptides of unmodified histone (T). Dashed lines: tryptic peptides from maloylated F2b histone (TM). Residues in parentheses (if not arranged alphabetically): probable sequence. Residues in parentheses (in alphabetical order): sequence remains to be determined.

Table II. Amino acid composition of 3 large tryptic peptides from maleyated F2b histone

| Amino acid | Peptide TM1 *M*% | R | TM3 *M*% | R | TM8 *M*% | R |
|---|---|---|---|---|---|---|
| Lysine | 30.0 | 9 | 10.2 | 4 | 17.5 | 5 |
| Histidine | – | – | 2.4 | 1 | 3.7 | 1 |
| Arginine | 6.9 | 2 | 2.6 | 1 | – | – |
| Aspartic acid | 3.5 | 1 | 10.3 | 4 | – | – |
| Threonine | 3.2 | 1 | 2.5 | 1 | 10.9 | 3 |
| Serine | 6.6 | 2 | 12.5 | 5 | 10.5 | 3 |
| Glutamic acid | 6.8 | 2 | 7.8 | 3 | 7.4 | 2 |
| Proline | 13.6 | 4 | 2.6 | 1 | 3.8 | 1 |
| Glycine | 6.8 | 2 | 5.2 | 2 | 7.1 | 2 |
| Alanine | 17.0 | 5 | 2.5 | 1 | 10.9 | 3 |
| Valine | 6.8 | 2 | 12.4 | 5 | 7.2 | 2 |
| Methionine | – | – | 5.0 | 2 | – | – |
| Isoleucine | – | – | 8.0 | 3 | – | – |
| Leucine | – | – | 2.5 | 1 | 14.6 | 4 |
| Tyrosine | – | – | 7.5 | 3 | 6.8 | 1 |
| Phenylalanine | – | – | 5.3 | 2 | – | – |

Amino acids are expressed as mole percents of all amino acids recovered. All serine values were corrected (10%) for hydrolytic losses. *M*%, mole percent; R, number of residues.

From the amino acid composition of these 3 large TM peptides (Table II) can be concluded that peptide TM1 originated from the $NH_2$ terminus, peptide TM3 represents the central portion containing methionine and phenylylanine (core), and peptide TM8 is the COOH terminal fragment. Digestion of the cyanogen bromide fragments and of the maleylated peptides with chymotrypsin (Worthington) and with thermolysin (CalBiochem) resulted in a number of overlapping peptide species. Partial identification and amino acid sequence analysis of such peptides isolated by chromatography on Dowex 50W resin and by gel filtration on Sephadex G-25 (a detailed report will be published elsewhere) permitted reconstruction of a large portion of the amino acid sequence of the F2b histone (Figure).

In accord with the reports of other investigators describing assymetrical distribution and clustering of basic amino acids in the arginine-rich histone F2al (ref. [1,12])

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and in the very lysine-rich histone F1 (ref. [2,3]), the moderately lysine-rich histone F2b, studied in our laboratory, shows a similar assymetry. Most of the neutral and hydrophobic amino acids are situated in the central part of this protein (i.e., peptides T3 or T11, T16, and T21 contain 31 of the 94 nonbasic residues present in F2b histone). Regions on either side of the nonbasic center are relatively enriched in basic amino acids with the $NH_2$ terminal region being definitely more basic (14 of the 33 residues are basic amino acids) than the COOH terminal portion (12 of the 54 amino acids are basic residues). The biological significance of the observed assymetry in amino acid distribution in the F2b histone from calf thymus is not clear[13].

*Zusammenfassung.* Die Aminosäuresequenz der Histonfraktion F2b aus Kalbsthymus wird angegeben und die Verteilung der basischen Aminosäuren als nicht symmetrisch ermittelt.

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## Stereospecificity of the Prostaglandin 15-Dehydrogenase from Swine Lung[1]

Recent studies of the metabolism of natural prostaglandins (PGs) have revealed a number of significant pathways commencing with either (1) saturation of the C-13 double bond[2], (2) $\beta$-oxidation of the carboxylic acid side chain[3,4], or (3) dehydrogenation at C-15 producing 15-keto PGs[2,5]. Of these, shortening of the carboxylic acid side chain[6] or oxidation of the hydroxy group at C-15 (present in all primary PGs) causes a marked decrease in smooth muscle stimulating, vasodepressor[7], and platelet aggregation inhibition properties[8].

The 15-dehydrogenase from the high speed supernatant of swine lung is the only isolated PG metabolizing enzyme with a single chemical action. Anggård and Samuelsson[5] report that this enzyme effects 15-dehydrogenation of all PGs except those of the PGB type. The enzyme is NAD dependent and highly specific for PGs – even 15(S)-hydroxyeicosa-8,11,13-trienoic acid is inert[5].

Diastereomeric PGs and a wide variety of non-acidic prostane derivatives are now available by efficient total syntheses[9–12] and a preliminary examination of the activity of these substances on 4 different pharmacological preparations has revealed unexpected biological potency for some of the unnatural diastereomers[13]. This communication is a preliminary account of the study of the stereostructural requirements (for substrate) of the swine lung dehydrogenase using synthetic substrates with the object of determining whether structure modifications would reduce the rate of biological degradation without reducing the pharmacological activity and thus serve as an indication of the modifications needed to produce substances having long-lasting actions like those of the natural PGs.

*Materials and methods.* The swine lung 15-hydroxyprostaglandin dehydrogenase was isolated by a slight modification of the published method[5]. Dehydrogenation rates with *nat*-$PGE_1$[14] (Km = 5.3 $\mu M$) were comparable to those reported (5.6 $\mu M$)[5]. The formation of 15-keto-prostanes was followed spectrophotometrically using the 340 nm absorption of the NADH generated in the reaction[5]. In the case of $PGE_1$-diastereomers the amount of 15-keto-$PGE_1$ formed could be confirmed spectrophotometrically using the strong, but transient, absorption that develops at 500 nm on base treatment. In addition the presence of the 15-keto products was confirmed by thin-layer chromatographic (TLC)[15] comparison with authentic samples prepared by chemical oxidation (using dicyano-dichlorobenzoquinone).

*Results and discussion.* Typical time courses of the enzymic dehydrogenation of *nat*-$PGE_1$ and the 4 racemic isomers are shown in Figure 1[16]. The maximum $\Delta A_{340}$ values obtained are generally 75–95% of those expected based on the generation of a stoichiometric amount of NADH, and are proportional to the amount of *nat*-$PGE_1$ used over a 10-fold range in concentration (5–66 $\mu M$). The amount of NADH decreases slowly after the maximum value has been reached, suggesting the presence of a NADH-dependent enzyme system (and substrate?) since the amount of 15-keto-$PGE_1$ does not diminish in the manner of the 500 nm absorption. For this reason direct assays of 15-keto-$PGE_1$ are more reliable for substrates reacting slowly.

Figure 1 clearly indicates that racemic $PG(E\alpha\alpha)_1$ and $PG(E\beta\alpha)_1$ are dehydrogenated at half molar amounts of *nat*-$PG(E\alpha\alpha)_1$ indicating that only one antipode is used.

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