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Gene organization and primary structure of human hormone-sensitive lipase: Possible significance of a sequence homology with a lipase of *Moraxella* TA144, an antarctic bacterium

(adipose tissue/lipid metabolism/psychrotroph)

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ABSTRACT **The human hormone-sensitive lipase (HSL) gene encodes a 786-aa polypeptide (85.5 kDa). It is composed of nine exons spanning ≈11 kb, with exons 2–5 clustered in a 1.1-kb region. The putative catalytic site (Ser^{423}) and a possible lipid-binding region in the C-terminal part are encoded by exons 6 and 9, respectively. Exon 8 encodes the phosphorylation site (Ser^{551}) that controls cAMP-mediated activity and a second site (Ser^{553}) that is phosphorylated by 5′-AMP-activated protein kinase. Human HSL showed 83% identity with the rat enzyme and contained a 12-aa deletion immediately upstream of the phosphorylation sites with an unknown effect on the activity control. Besides the catalytic site motif (Gly-Xaa-Ser-Xaa-Gly) found in most lipases, HSL shows no homology with other known lipases or proteins, except for a recently reported unexpected homology between the region surrounding its catalytic site and that of the lipase 2 of *Moraxella* TA144, an antarctic psychrotrophic bacterium. The gene of lipase 2, which catalyses lipolysis below 4°C, was absent in the genomic DNA of five other *Moraxella* strains living at 37°C. The lipase 2-like sequence in HSL may reflect an evolutionarily conserved cold adaptability that might be of critical survival value when low-temperature-mobilized endogenous lipids are the primary energy source (e.g., in poikilotherms or hibernators). The finding that HSL at 10°C retained 3- to 5-fold more of its 37°C catalytic activity than lipoprotein lipase or carboxyl ester lipase is consistent with this hypothesis.**

Adipose tissue triacylglycerol is the quantitatively most important source of stored energy in mammals. Hormone-sensitive lipase (HSL; EC 3.1.1.3) has a critical role in the control of energy homeostasis by catalyzing the hydrolysis of adipocyte triacylglycerol and thereby releasing free fatty acids for transport to energy-requiring tissues. Like glycogen phosphorylase, the corresponding and much more well-known enzyme in carbohydrate metabolism, HSL is under acute neural and hormonal control. HSL is activated by catecholamines through the cAMP-dependent phosphorylation of a single serine residue (1, 2). The antilipolytic effect of insulin, one of the most important acute actions of the hormone, is mediated by the prevention of this phosphorylation (3).

The key metabolic role of HSL is accentuated in certain physiological states. During prolonged periods of starvation, as when birds are migrating or during winter for hibernators and polar animals, mobilization of adipose tissue lipids becomes important for survival. An adaptative enhancement of HSL activity is required under these conditions. In humans, variations of HSL have been proposed to take place in various situations (4, 5). However, the mechanisms for the long-term regulation of HSL mRNA and protein levels have not yet been studied.

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HSL has some unique features among lipases. Unlike lipoprotein lipase, the rate-limiting enzyme of triacylglycerol storage in adipose tissue, HSL does not belong to the so-called lipase gene family that also includes hepatic lipase and pancreatic lipase (6, 7). The rat HSL primary structure showed no homology with any other known lipases or proteins besides the putative catalytic site motif (Gly-Xaa-Ser-Xaa-Gly, where the active serine is underlined) found in most lipases (6). Moreover, HSL is the only triacylglycerol lipase in which the activity is controlled through phosphorylation. In addition, HSL has the same catalytic activity toward both cholesterol ester and triacylglycerol and may have an important role in steroidogenesis in ovaries, adrenal cortex, testes, and placenta (6, 8).

In view of the key role of HSL in energy metabolism, its unique properties when compared to other lipases, and its possible role in pathophysiological states such as diabetes and obesity in which an alteration of free fatty acid mobilization may be involved, we considered it important to analyze the structural, genetic, and regulatory features of human HSL. The combined knowledge of the gene structure and of its localization to chromosome 19 cent–q13.3 (6) would clearly be important for future genetic studies in humans. We have therefore cloned and characterized the human HSL gene† and below report its organization and the deduced amino acid sequence of the human HSL protein. Recently, the region surrounding the putative catalytic site of the lipase 2 (EC 3.1.1.3) of *Moraxella* TA144 (*M.*TA144), an antarctic psychrotrophic bacterium, was reported to be homologous to the corresponding region of HSL (9). We have attempted to find an explanation for this unexpected homology. *M.*TA144 lipase 2 normally catalyzes hydrolysis of lipids at a very low temperature. It seemed possible that such cold adaptability could be an important property also for HSL since mobilization of endogenous lipids at low temperatures for energy production would be of critical survival value in certain species (e.g., poikilotherms and hibernators). We have therefore determined whether the homology is specific for cold-adapted strains of *Moraxella* and whether HSL catalyzes hydrolysis more efficiently at low temperatures than some lipases that are not involved in endogenous lipid mobilization.

EXPERIMENTAL PROCEDURES

cDNA and Genomic Clone Isolation and Sequencing. One million recombinant phage from a human fat cell λgt11 cDNA library were screened using a rat adipose tissue full-length

Abbreviations: HSL, hormone-sensitive lipase; *M.*TA144, *Moraxella* TA144.

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. L11706).

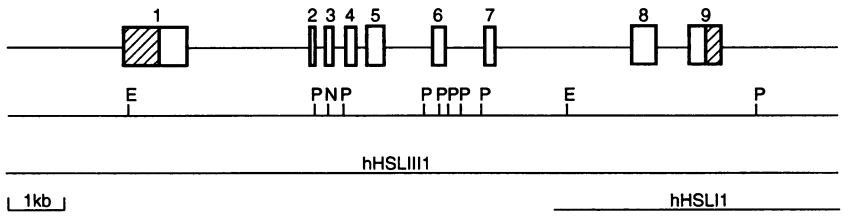

FIG. 1. Organization of the human HSL gene. Exons (boxes 1–9) and intronic regions are shown above a restriction map (E, *Eco*RI; N, *Not* I; P, *Pst* I). Noncoding regions are represented by hatched areas. The regions spanned by the two cosmid clones used to characterize the gene are also shown.

cDNA probe (6). The largest clone (1.4 kb) contained the sequence encoding the putative catalytic and phosphorylation sites, as well as a putative polyadenylylation signal. This fragment was subcloned into M13 phage vectors and sequenced by the dideoxynucleotide chain-termination method using a modified T7 DNA polymerase (United States Biochemical).

This cDNA clone was used to screen 600,000 recombinant cosmids from a human pWE15 genomic library (Stratagene). One of the two clones found to hybridize (hHSLIII1) spanned the entire gene. Exons were localized to particular restriction fragments of cosmid inserts by Southern blot analyses, for which rat or human cDNA pieces were used as probes. After subcloning these restriction fragments into pBluescript II vectors (Stratagene) and completely sequencing the exons and adjacent regions, exon–intron boundaries were determined for the last four exons by comparison with the human cDNA sequence. The open reading frames of rat (6) and human cDNA sequences showed a high homology (83%) and exon–intron boundaries of the first five exons could therefore be determined by comparison with the rat adipose tissue cDNA sequence. The validity of these boundaries was confirmed without ambiguity by the comparison between the genomic sequence and the sequence of two overlapping human testis cDNA clones corresponding to the coding region of the gene (L.S.H. and Gudrun Fredrikson, unpublished data). Intron sizes were determined by restriction map analyses and PCR amplification of the introns.

For primer-extension analysis, an end-labeled 21-mer antisense oligonucleotide that maps to 130 nt downstream from nt +1 was hybridized to 10 μg of total RNA from human adipose tissue and extended with a modified Moloney murine leukaemia virus reverse transcriptase (Superscript RNase H⁻; GIBCO/BRL) for 90 min at 42°C. PCR amplification of human adrenal and adipose tissue cDNAs (QuickClone; Clontech) was used to confirm the organization of the first exon, which contains a long 5′ untranslated sequence (data not shown).

Data base searches, alignment of protein sequences, and prediction of secondary structure for the human HSL protein were performed using the GENEPRO sequence analysis software (Riverside Scientific, Bainbridge Island, WA).

Southern Blot Analysis of *Moraxella* Strain Genomic DNAs. Genomic DNA was prepared from five *Moraxella* strains (Culture Collection, University of Göteborg, Sweden) grown in Todd Hewitt medium (Difco). The DNA was digested with *Eco*RI or *Bam*HI and transferred to nylon membrane. For comparison, genomic DNA from *M.*TA144 (9) was digested and transferred to the same membrane. A 347-nt sequence was PCR-amplified using *M.*TA144 genomic DNA with a forward 27-mer primer that corresponds to nt 670–696 from the open reading frame of the *M.*TA144 lipase 2 gene and a 27-mer reverse primer that corresponds to nt 991–1017 of the same gene (9). The PCR product was purified, labeled, and hybridized at 42°C to *Moraxella* genomic DNA. The membrane was washed in 30 mM NaCl/3 mM sodium citrate/0.1% SDS at 50°C and analyzed using a Fujix BAS 2000 BioImaging analyzer.

Assay of Lipase Activity. The influence of temperature on the catalytic activity of several lipases was investigated using *p*-nitrophenylbutyrate as a substrate (10). *p*-Nitrophenylbutyrate shows little specificity and is hydrolyzed by a wide range of lipases. Lipoprotein lipase (EC 3.1.1.34) and carboxyl ester lipase (EC 3.1.1.13), which hydrolyze, respectively, triacylglycerols and cholesterol esters, were selected for comparison with HSL. Purified human pancreatic carboxyl ester lipase (11), rat HSL (2), and bovine lipoprotein lipase (12) were incubated in 1 ml of incubation buffer (0.1 M NaH_2PO_4/0.9% NaCl/0.5 mM *p*-nitrophenylbutyrate) for 7 min at various temperatures. Optimal conditions were obtained by adding 1 mM dithioerythritol for HSL assay and 1.5 international units of heparin for the lipoprotein lipase assay and by adjusting the pH to 7.25 for the carboxyl ester lipase and HSL assays and to 7.4 for the lipoprotein lipase assay. Enzyme reactions were terminated by the addition of 3.25 ml of methanol/chloroform/heptane, 10:9:7 (vol/vol), to form a

Table 1. Exon sizes and sequence at intron–exon boundaries of the HSL gene

Exon	Exon length, bp	5′ splice donor	Intron length, bp	3′ splice acceptor	Codon phase	Amino acid(s)
1	1144	TTCCAG^{1144}GTGAGC	≈2100	CCACAG^{1145}TTCACG	0	Gln/Phe^{172}
2	91	GCCTCAG^{1235}TGAGT	110	CCCTAG^{1236}GTGTGG	I	Ser^{202}
3	146	CTATCGG^{1381}TGAGG	183	CTCCAG^{1382}TCTCTG	0	Ser/Ser^{251}
4	186	GGACACG^{1567}TAGG	137	CCCAG^{1568}GACAGT	0	Gln/Asp^{313}
5	295	TCCTTGG^{1862}TGAGC	≈800	AACCAG^{1863}GCTCAA	I	Gly^{411}
6	228	ATGCTGG^{2090}TGGTG	≈620	CTGCAG^{2091}GTGCAA	I	Gly^{487}
7	177	TAGCAGG^{2267}TGAGT	≈2400	TCTCAG^{2268}AGCCGA	I	Glu^{546}
8	425	ATCGTGG^{2692}TGAGC	≈520	CCGTAG^{2693}GCGTGC	0	Val/Ala^{688}
9	558	TAATTA^{3250} end			—	—

Exon and intron sequences are in large and small uppercase type, respectively. Invariant nucleotides at the splice sites are underlined and the locations of the splice junctions are indicated. Intron phases I and 0 refer to introns that interrupt after the first and third nucleotide of a codon, respectively. The amino acid(s) encoded at the splice sites are indicated.

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  1 MDLRTMTQSL VTLAEDNIAF FSSQGPGETA QRLSGVFAGV REQALGLEPA LGLLGVAHLF DLDPETPANG YRSLVHTARC
 81 CLAHLLHKSR YVASNRRSIF FCTSHNLAEL EAYLAALTQL RALVYYAQRL LVTNRPGVLF FEGDEGLTAD FLREYVTLHK
161 GCFYGRCLGF QFTPAIRPFL QTISIGLVSF GEHYKRNETG LSVAASSLFT SGRFAIDPEL RGAEFERITQ NLDVHFWKAF
241 WNITEMEVLS SLANMASATV RVSRLLSLPP EAFEMPLTAD PTLTVTISPP LAHTGPGPVL VRLISYDLRE GQDSEELSSL
321 IKSNGQRSLE LWPAPQQAPR SRPLIVHFHG GGFVAQTSRS HEPYLKSWAQ ELGAPIISID YSLAPEAPFP RALEECFFAY
401 CWAIKHCALL GSTGERICLA GDSAGGNLCF TVALRAAAYG VRVPDGIMAA YPATMLQPAA SPSRLLSLMD PLLPLSVLSK
481 CVSAYAGAKT EDHSNSDQKA LGMMGLVRRD TALLLRDFRL GASSWLNSFL ELSGRKSQKM SEPIAEPMRR SVSEAALAQP
                                                                              • o
561 QGPLGTDSLK NLTLRDLSLR GNSETSSDTP EMSLSAETLS PSTPSDVNFL LPPEDAGEEA EAKNELSPMD RGLGVRAAFP
641 EGFHPRRSSQ GATQMPLYSS PIVKNPFMSP LLAPDSMLKS LPPVHIVACA LDPMLDDSVM LARRLRNLGQ PVTLRLVEDL
721 PHGFLTLAAL CRDGPGRRAV RGAHPPRPHS SRRSRAERGD GGCGGRRGLR GATLKACCSH LRRPPS*  786
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FIG. 2. Deduced amino acid sequence of human HSL. The putative catalytic site containing the active serine is underlined. The serine residue (regulatory site) phosphorylated by cAMP-dependent protein kinase is indicated by a solid circle and a second phosphorylation site (basal site) is indicated by an open circle. A possible lipid binding site is overlined.

two-phase partition system. The absorbance of the supernatant containing *p*-nitrophenol was measured at 400 nm.

RESULTS

Structure of the HSL Gene. Two overlapping human genomic clones were isolated and characterized (Fig. 1). The predicted restriction map of the gene was in agreement with results obtained by Southern blot analyses of human genomic DNA (unpublished data), indicating that rearrangements or deletions did not occur during the cloning of the DNA.

The human HSL gene is composed of nine exons spanning ≈11 kb. The sequences bordering the splice-site junctions of each intron follow the GT/AG rule (Table 1). The transcriptional start site was determined by primer-extension analysis of the 5′ end of human HSL mRNA (data not shown) and was found to be 632 nt upstream from the translational start site. The first and largest exon encodes the entire 5′ untranslated sequence and the coding sequence for the first 171 aa residues. Exons 2–5, with lengths ranging from 91 to 295 nt, are clustered in a 1.1-kb genomic region. The putative catalytic site and the phosphorylation sites are encoded by exons 6 and 8, respectively. A possible lipid binding site (13) is encoded in the ninth and final exon, which also contains the 3′ untranslated region and a putative polyadenylylation signal.

Primary Structure of Human HSL. The deduced amino acid sequence of human HSL is presented in Fig. 2. The 786-aa protein has a calculated molecular weight of 85,500 in the absence of posttranslational modifications. This value is consistent with the apparent size determined by SDS/PAGE, which indicates a slightly larger size for the human protein than for the rat HSL (88 kDa vs. 84 kDa) (14). An error was recently detected in the rat HSL cDNA sequence that alters the C-terminal part of the enzyme by shifting the open reading frame and extending the length of the protein by 11 aa (Z. Li, M. Sumida, A. Birchbauer, M. C. Schotz, and K. Reue, personal communication). By taking this modification into account, the amino acid sequences of the rat and human enzymes show 83% identity and 86% similarity, including 22 conservative substitutions.

The phosphorylation site sequence (Met-Arg-Arg-Ser^{551}-Val-Ser^{553}-Glu-Ala-Ala) is completely conserved in bovine (15), rat (6), mouse (Li *et al.*, personal communication), and human HSLs (Fig. 3). The region surrounding these residues is predicted to be fairly hydrophilic and α-helical, consistent with a position at the surface of the protein accessible to the protein kinases (16). The human HSL differs from the rat sequence in the region immediately upstream of the phosphorylation sites. The 12 aa deleted in human HSL modifies the HSL secondary structure since a connecting loop present in rat HSL between a hydrophilic region and the α-helix containing the phosphorylation sites is not found in human HSL. Interestingly, the deleted sequence is encoded by the 3′ end of exon 7 whereas the phosphorylation sites are encoded by exon 8. This deletion was found in several cDNA and genomic clones from different libraries ruling out the possibility of a cloning artifact. A single amino acid deletion corresponding to Gln^{53} in the rat sequence and two insertions (Ser^{328} and Thr^{585}) are found in human HSL when compared with rat HSL. Finally, the larger size of the human protein is due to a longer C-terminal region.

Analysis by the method of Kyte and Doolittle (16) predicts several relatively hydrophobic regions. One of them includes a sequence corresponding to the putative catalytic site motif found in most lipases (Fig. 2). Another, located in the C-terminal region, contains an 8-aa sequence representing a possible lipid binding site by analogy with a similar sequence in the cholesteryl ester transfer protein and other proteins binding nonpolar lipids such as the lecithin:cholesterol acyltransferase and the pancreatic carboxyl ester lipase (13).

Comparison of HSL with *M.*TA144 Lipase 2 and Other Proteins. A computer search of GenBank, EMBL, and Protein Identification Resource libraries (March 1992) failed to reveal any significant homology between HSL and any other eukaryotic proteins. In particular, HSL shared no homology with the members of the lipase gene family including pancreatic lipase, hepatic lipase, and lipoprotein lipase (7) or with any other known eukaryotic lipases. However, an unexpected homology was recently reported between HSL and the lipase 2 of *M.*TA144 (9), an antarctic psychrotrophic bacterium (Fig. 4). This homology spans 89 residues showing 42% identity and 52% similarity. The sequence contains two

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                                                    ↓   • o
Human HSL 524 SWLNSFLELSGRKSQKMSE.............PIAEPMRRSVSEAALAQP 560
              :::::::::::::   :                 : ::::::::::::::
  Rat HSL 524 SWLNSFLELSGRKPHKTPVACNRDTAPHGFWALTESMRRSVSEAALAQP 572
                                                    ↑   • o
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FIG. 3. Alignment of the amino acid sequences upstream of the phosphorylation sites of human and rat HSL. The regulatory and basal phosphorylation sites are indicated by solid and open circles, respectively. The boundary between the amino acid sequences encoded by exons 7 and 8 is indicated by arrows. Identical amino acids are indicated by colons.

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                                                                    * * *
Human HSL 345 IVHFHGGGFVAQTSRSHEPYLKSWAQELGAPIISIDYSLAPEAPFPRALEECFFAYCWAIKHCALLGSTGERICLAGDSAGGNLCFTVA 433
              . ::::::      .: . .    : ..:.::  ::: : : :: .:  :: :   :   :: .  :: : :::::: :   ::
Lipase 2  161 MLFFHGGGFCIGDIDTHHEFCHTVCAQTGWAVVSVDYRMAPEYPAPTALKDCLAAYAWLAEHSQSLGASPSRIVLSGDSAGGCLAALVA 249
                                                                    * * *
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FIG. 4. Alignment of the conserved domains of human HSL and *M.*TA144 lipase 2. The conserved His-Gly dipeptide is underlined. The putative catalytic site motif containing the active serine is indicated by stars. Identical amino acids and conservative substitutions are indicated by colons and periods, respectively.

highly conserved regions, the putative catalytic site and 6 aa surrounding the dipeptide His-Gly. This dipeptide is found in most, but not all lipases, 70–100 aa upstream of the putative catalytic serine (17).

To assess whether the lipase 2 gene was specific to cold-adapted *Moraxella* strains, a 347-nt PCR fragment of the lipase 2 gene was hybridized to genomic DNA from five *Moraxella* strains living at body temperature. Although the fragment hybridized strongly to genomic DNA from *M.*TA144, no hybridization was observed with genomic DNA from *Moraxella catarrhalis, Moraxella nonliquefaciens, Moraxella osloensis, Moraxella lincolnii*, and *Moraxella atlantae* (Fig. 5).

Effect of Temperature on the Catalytic Activity of HSL. Since lipase 2 efficiently hydrolyzes substrates at a low temperature (9), we determined whether HSL had a similar property (Fig. 6). When compared to enzyme-mediated hydrolysis of *p*-nitrophenylbutyrate at 37°C, HSL showed a relatively higher activity at low temperatures than carboxyl ester lipase or lipoprotein lipase, two enzymes that are not involved in endogenous lipid mobilization. The percentages of 37°C activity retained at 10°C were 25–35% for carboxyl ester lipase, 10–15% for lipoprotein lipase, and 70–80% for HSL (n = 5).

DISCUSSION

Each putative functional region of HSL is encoded by a different exon (Figs. 1 and 2), raising the possibility that the enzyme is the result of the assembly of different domains during evolution. In particular, exon 6 encoding the putative catalytic site is flanked by phase I introns interrupting glycine codons (Table 1) as described for modules of mosaic proteins (18). This module may have been shuffled to its current position in the HSL gene. The identical phasing of its introns could have led to the coupling of adjacent exons through the process of intronic recombination by preserving a continuous reading frame. Data base searches were performed to examine the possible links between HSL and other proteins. The sequence surrounding the putative catalytic site is consistent with the motif (β-strand, type II' β-hairpin with serine in the ε-conformation,α-helix) proposed to be common to triacylglycerol lipases and esterases (19). However, the sequence of exon 6, which encodes the putative catalytic site, showed no homology with the corresponding exons of other mammalian lipase genes. Moreover, a detailed analysis of the organization of the genes failed to reveal any relationship between the HSL gene and any known lipase gene family.

An analogous conclusion was drawn from the comparison of exon 8 encoding the phosphorylation sites with phosphorylatable protein genes. The regulatory site phosphorylated by cAMP-dependent protein kinase (15) was identified as Ser^{551}. Ser^{553} has been found to be a second phosphorylation site, termed the basal site since it is phosphorylated by the 5'-AMP-activated protein kinase without concomitant increase of enzyme activity (20) but known to prevent the phosphorylation of the regulatory site and vice versa. It is not known whether the deletion in human HSL upstream of the phosphorylation sites modifies its functional characteristics (Fig. 3). It could possibly lead to a different accessibility of the phosphorylation sites to the protein kinases and/or a modulation of the effect of the phosphorylation on HSL activation, as shown for acetyl-CoA carboxylase (21). In one of the different forms of acetyl-CoA carboxylase coexisting *in vivo*, an insertion of eight extra amino acids located upstream of the phosphorylation site inhibits the phosphorylation by cAMP-dependent protein kinase. On the other hand, the deletion in the HSL sequence occurs at the 3' end of exon 7, a region that could represent a link between two structural domains of the protein. It is therefore possible that this connective loop could be shortened without any change of the function of the protein.

The combined knowledge of the organization of the human HSL gene and its location on chromosome 19 q13.1 (6, 22) has an obvious potential for clinically oriented research. Interestingly, another gene involved in lipid metabolism, the gene encoding transcription factor C/EBPα, also maps to band

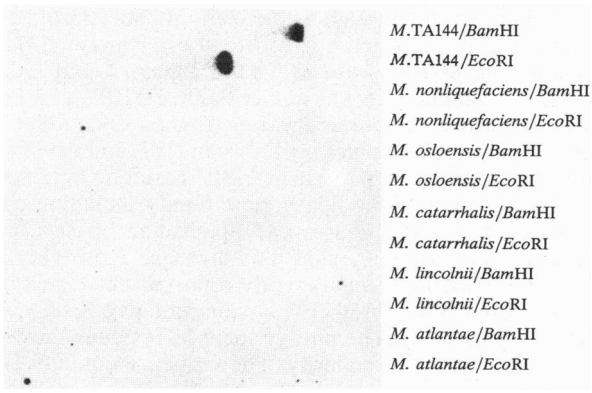

FIG. 5. Southern blot analysis of six *Moraxella* strain genomic DNA. A PCR-amplified fragment of the *M.*TA144 lipase 2 gene was hybridized to *Moraxella* genomic DNA digested with *Eco*RI or *Bam*HI. *M.*TA144 was isolated from antarctic sea water; *M. catharralis* and *M. nonliquefaciens* were from the nasal cavity; *M. osloensis* was from the cerebrospinal fluid; *M. lincolnii* was from the nasopharynx; *M. atlantae* was from blood. Except for *M.*TA144, all strains were isolated from human tissues.

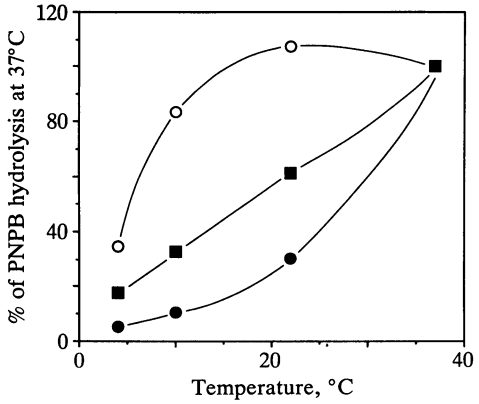

FIG. 6. Hydrolysis of *p*-nitrophenyl butyrate (PNPB) by HSL (○), lipoprotein lipase (●), and carboxyl ester lipase (■). The values are expressed as the percentages of the catalytic activity showed at 37°C. Carboxyl ester lipase (0.18 μg), lipoprotein lipase (5 μg), and HSL (0.1 μg) were incubated for 7 min in the presence of 0.5 mM PNPB at the indicated temperatures. No enzyme was added in the blanks analyzed in parallel. Each point is the mean of duplicates. The data shown are representative of three experiments.

q13.1 (23). The possible involvement of the HSL gene in inherited lipid metabolism disorders such as lipodystrophy can now be investigated with a combination of linkage studies and search for mutations or rearrangements of the gene. Several familial disorders (e.g., myotonic dystrophy and malignant hyperthermia) have been mapped to this region of chromosome 19. Although it has been proposed, the involvement of the HSL gene in these diseases has recently been ruled out (22, 24, 25).

In species with specialized lipid stores (i.e., in most vertebrates), a hormonally and/or neuronally controlled lipase is required to provide the precise adjustment of lipid mobilization necessary to satisfy the continuous fluctuations of the energy demand. In accordance with this, the presence of HSL or a similar enzyme, controlled by cAMP-dependent phosphorylation and activation, has recently been demonstrated in fish, reptilian, and amphibian adipose tissue (26). HSL should be particularly crucial for survival in poikilotherms in situations characterized by strict dependence on endogenous lipid mobilization at low temperatures (e.g., in fishes during spawning or in amphibians during winter). Such cold adaptability could be a definite survival advantage also in mammals, as illustrated by hibernators. During winter, hibernators have a body temperature of ≈5°C and rely almost completely on fat stores as the energy source, with elevated plasma free fatty acid levels (27). During the nonshivering phase of periodic arousals, triacylglycerols are hydrolyzed in brown adipose tissue to produce heat (28, 29). This situation requires a highly active HSL acting at a low temperature. A dramatic increase of HSL mRNA levels was indeed recently reported during marmot hibernation (27). Cold adaptability may thus be a much more important property for HSL than for other lipases (e.g., those involved in dietary lipid digestion and metabolism).

In this context, we have considered the possibility that the unique homology between HSL and the *M*.TA144 lipase 2 (Fig. 4) reflects a common cold adaptability. The findings that the homologous region was limited to the psychrotrophic *M*.TA144 strain (Fig. 5) and that HSL showed a 3- to 5-fold higher catalytic activity at a low temperature than lipoprotein lipase or carboxyl ester lipase (Fig. 6) are consistent with this hypothesis. Some structural features of HSL and lipase 2 may also be of interest in connection with this. An appropriate folding flexibility is important for catalyzing lipolysis at a low temperature and could be achieved by a relatively high number of small-sized amino acids that are known to be involved in peptide chain β-turn structures. The stack of glycine residues after the conserved His-Gly dipeptide of HSL and lipase 2 (Fig. 4) is not found in other lipases and could be important in this respect (9). The three-dimensional structure of pancreatic lipase predicts that the region surrounding the His-Gly dipeptide constitutes one of the hydrophobic "wings" flanking the catalytic site (30). The contact between the triacylglycerol substrate and the active serine probably requires a conformational change in this segment (30). Since the distance between the catalytic site and the dipeptide is rather constant among lipases (17), the dipeptide could, therefore, constitute a hydrophobic region flanking also the HSL catalytic site. If so, the presence of several glycine residues could provide more flexibility to the flanking "wing" and facilitate lipase 2 and HSL action at low temperatures.

Clearly, more experimental evidence (e.g., by site-directed mutagenesis studies) is needed to corroborate the suggested explanation for the homology between HSL and the *M*.TA144 lipase. The elucidation of the human HSL gene organization and primary structure, in combination with the previously reported chromosomal localization, should prove useful for the examination of the transcriptional regulation and for the evaluation of a possible involvement of the human HSL gene in inherited disorders of lipid metabolism.

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