Characterization and Expression of L-Amino Acid Oxidase of Mouse Milk*

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L-Amino acid oxidase (LAO) was purified from mouse milk. LAO reacted with L-amino acids in an apparent order of Phe > Met, Tyr > Cys, Leu > His >> other 11 amino acids tested and produced H₂O₂ in a dose- and time-dependent manner. LAO in milk had a molecular mass of about 113 kDa and was converted to a 60-kDa protein by SDS-PAGE. LAO consisted of two subunits. The N- and C-terminal amino acid sequence determination followed by cDNA cloning showed that the 60-kDa protein consisted of 497 amino acids. LAO mRNA spanned about 2.0 kb, and its expression was found only in the mammary epithelial cells. Glucocorticoid was essential for LAO gene expression. Thus, the LAO gene is expressed acutely upon the onset of milk synthesis. LAO mRNA increased 1 day before parturition, peaked during early to mid-lactation, and decreased at the end of lactation. This is the first demonstration showing that LAO is present in milk. Mastitis is caused by an intramammary bacterial infection. As mouse milk produced H₂O₂ using endogenous free amino acids, we suggest that LAO, together with free amino acids, is responsible for killing bacteria in the mammary gland.

The mammary gland starts milk synthesis at the end of pregnancy to supply milk to the newborn neonate. Milk contains vital nutrients such as proteins, carbohydrates, lipids, minerals, and vitamins together with bioactive substances including immunoglobulins, bioactive peptides, peptide and steroidal hormones, and growth factors. Antibacterial factors including immunoglobulins, bioactive peptides, peptide and steroidal hormones, and growth factors are present in milk as well (5, 6). The presence of these substances in milk may confer the biological effect on both the mother and her offspring’s survival. Some of these substances begin to be synthesized upon the onset of milk synthesis in the mammary gland. It is well established that prolactin (PRL)³ is a key hormone to regulate lactose and casein synthesis. PRL is able to stimulate the mammary gland at the end of pregnancy since the PRL receptor gene is expressed half a day before parturition (7). To find the genes expressed highly in the late pregnant mouse mammary gland, mRNA screening was carried out using differential display methods. One cDNA fragment was obtained, and the entire nucleotide sequence from the 5′- to 3′-end was determined in the present experiments. The nucleotide sequence of this cDNA has high similarity to those for snake venom L-amino acid oxidase (LAO) (8), snake venom Apoxin 1 (9), and Fig1 protein in the mouse B cell (10). LAO catalyzes the oxidative deamination of particular L-amino acids (i.e. Cys, Phe, Met, Leu, Ile, Pro, and Tyr) and converts them into hydrogen peroxide (H₂O₂), ammonia, and keto acids (11). It has been demonstrated that administration of LAO isolated from snake venom results in the depletion of murine plasma amino acids such as Phe, Leu, Tyr, Met, Ile, and Val (12, 13). As a marker of both the H₂O₂ production and the amino acid conversion, we intended to isolate LAO from mouse milk.

In 1963, Armstrong and Yates (14) reported on concentrations of free amino acids in human and cow milk and showed that the concentrations of most free amino acids (except Glu) in milk are lower compared with those of the serum amino acids of the mothers who produce the milk samples. The lactating mammary gland almost equally absorbs all kinds of free amino acids from the circulating plasma in the rat (15) and sow (16). Except Cys, Gly, and Leu, the milk of a human, baboon, rhesus monkey, horse, cow, and pig contains almost equal amounts of amino acids presented mostly as the protein constituents (17). Although the concentrations of individual free amino acids in milk are not equally dependent upon the stage of lactation in the human (18) and sow (19), the imbalance of free amino acids in milk has similarly been observed in a number of species (20).

As compared with the amino acid compositions of mouse caseins (21), it is evident that free amino acids of LAO-convertible species (12, 13, 22) are few in mouse milk (20). The presence of LAO in milk remains unknown, but our hypothesis is that the imbalance of the free amino acid composition of milk is due to the LAO-catalyzed reaction of particular amino acids in the mammary gland.

Here we demonstrate that mouse milk contains LAO and prove our hypothesis in the present experiments. The physiologically important role of LAO in milk is also discussed.

EXPERIMENTAL PROCEDURES

Reagents and Chemicals—α-Dianisidine was obtained from Tokyo Kasei (Tokyo, Japan); horseradish peroxidase, the amino acid standard solution (Type H), and oxytocin were from Wako Pure Chemicals (Osaka, Japan); the silver staining kit and SDS-PAGE molecular weight markers were purchased from Bio-Rad; Immobilon was from Millipore Japan (Tokyo, Japan); lysylendopeptidase was from Roche.

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The abbreviations used are: PRL, prolactin; LAO, L-amino acid oxidase; r, correlation coefficient; RACE, rapid amplification of cDNA ends; DIG, digoxigenin; HPLC, high pressure liquid chromatography; nt, nucleotides; MOPS, 4-morpholinepropanesulfonic acid.
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Diagnostics; 5'- (version 2) and 3'-RACE systems, SuperScript II reverse transcriptase, oligo(dT) primer, and the RNA size marker were obtained from Invitrogen; EX Taq DNA polymerase was from TaKaRa (Kyoto, Japan); the TA cloning kit was from Invitrogen; the auto cycle sequencing kit and Hybond-N’ membrane were purchased from Amer sham Biosciences; 15% trichloroacetic acid and RNA were from Wako Pure Chemicals; Isogen was from Nippon Gene (Toyama, Japan); and steroids, amino acids, and the gel-filtration calibration kit were from Sigma. Primers were obtained from Sawady (Tokyo, Japan). All other chemicals and reagents were from Wako Pure Chemicals.

Animals—ICR-JCL mice were purchased from SLC (Shizuoka, Japan), maintained at 23 ± 1°C under a lighting schedule of 14 h (lights on 05:00–19:00 h), and given food and water ad libitum. The day on which a vaginal plug was found was designated as day 0 of pregnancy. The day of parturition was counted as day 0 of lactation. On day 13 of pregnancy, adenectomy, ovarietomy, and sham operations were done under pentobarbital anesthesia (25). One mg of cortisol or progesterone, dissolved in 0.1 ml of sesame oil, was administered subcutaneously at the completion of the operation (0 h).

Milk Sampling—Milk was collected from lactating mice on day 8 by mild suction under pentobarbital anesthesia. Oxytocin (0.1 unit) was injected intraperitoneally before milking. Skim milk was prepared by centrifugation at 10,000 × g for 10 min at 4°C. When prepared from skim milk, each sample was incubated with 0.1M sodium phosphate (pH 7.0) at 100,000 × g for 30 min at 4°C. All samples were stored at −50°C until use.

Determination of the LAO Activity—The LAO activity was determined by the production of H2O2. Horseradish peroxidase was dissolved in 0.1 M sodium phosphate (pH 7.0) at 40 mg/ml. The assay reagent was freshly prepared and consisted of 1 part o-dianisidine solution and 50 parts peroxidase solution. In the routine assay, L- and D-Leu (200 μM) were used as a positive and negative control, respectively. The sample was mixed with a 2.5-fold volume of the assay reagent. The incubation was carried out at 37°C for 120 min unless otherwise indicated in the text. The absorbance (A) was measured at 420 nm (A420) in a spectrophotometer. The LAO activity was expressed on the basis of the o-dianisidine oxidation (A420).

Purification of LAO—Proteins were separated on an Amersham Biosciences FPLC with Superose 12 (HR 16/50) at a flow rate of 0.6 ml/min. The elution buffer consisted of 0.5 M sodium phosphate (pH 7.0), 145 mM NaCl. The column was calibrated using a gel-filtration calibration kit. One ml of whey was applied, and every 1.2-ml fraction was collected. The proteins containing the LAO activity was dialyzed overnight against 20 mM Tris/HCl (pH 8.0). Ion-exchange chromatography was carried out using an AKTA prime on a RESOURCE™Q column (Amersham Biosciences) at a flow rate of 1 ml/min with each fraction set at 0.5 ml. The column was equilibrated with 20 mM Tris/HCl (pH 8.0), and the NaCl concentration was increased from 0 to 500 mM for the elution. The fraction containing the LAO activity was stored at −20°C.

The protein concentration was determined by measuring the A280 by the production of H2O2. The concentration was determined by measuring the A280 and A263, respectively. Total RNA was transcribed to cDNA at 42°C for 50 min in the presence of reverse transcriptase and primer using SuperScript II. With cDNA, EX Taq DNA polymerase, and primers, the PCR was cycled 35 times using a TaKaRa TP2000 thermal cycle. Each cycle consisted of denaturation at 94°C for 1 min, annealing at 57°C for 1 min, and extension at 72°C for 1 min.

SDS-PAGE and Amino Acid Sequencing—LAO was denatured in the presence of 6% SDS with 2-mercaptoethanol at 100°C for 5 min and separated by SDS-PAGE on a 7.5% polyacrylamide gel. The gel was fixed with 7% acetic acid, 20% methanol for silver staining. The molecular weight of LAO was estimated in comparison with those of SDS-PAGE molecular weight markers.

The denatured LAO was separated by SDS-PAGE and transferred onto Immobilon as described above. The protein band stained with Coomassie Blue was cut out, and amino acid sequence analysis was performed on a Procise 492 protein sequencing system (Applied Biosystems, Foster City, CA) under the pulse-liquid phase. For the fragmentation of LAO, the band was cut out from the gel stained with Coomassie Blue and digested with lysylendopeptidase for 18 h at 37°C. The peptide fragments were separated by HPLC on a Puresil C18 column (3.9 × 150 mm) (Waters, Milford, MA) with a linear gradient (0–60%) of 2-propanol:acetonitrile (7:3) in H2O containing 0.1% trifluoroacetic acid in 60 min at the flow rate of 1 ml/min. The amino acid sequence analysis of the peptide fractionated by HPLC was performed with a Procise RACE protein sequencing system.

Determination of the Amino Acid Content—Proteins in whey were precipitated by 5% trichloroacetic acid and removed by centrifugation at 10,000 × g for 30 min. Free amino acids and ammonia were analyzed using a Hitachi L-8500A amino acid analyzer (Tokyo, Japan) according to the manufacturer's instructions. To determine the conversion of free amino acids and ammonia were determined as described above.

Reverse Transcription-PCR and Nucleotide Sequencing—Total RNA was extracted from the third thoracic mammary gland using Isogen. All operations were done under nitrogen. The concentration and RNA integrity were measured by measuring the A260 and A230/A260, respectively. Total RNA was transcribed to cDNA at 42°C for 50 min in the presence of reverse transcriptase and primer using SuperScript II. With cDNA, EX Taq DNA polymerase, and primers, the PCR was cycled 35 times using a TaKaRa TP2000 thermal cycle. Each cycle consisted of denaturation at 94°C for 1 min, annealing at 57°C for 1 min, and extension at 72°C for 1 min.

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TABLE I

Amino acid contents of mouse milk (mean ± S.E., n = 5)

| I. Content | II. Decrease |
|------------|--------------|
| µM         | %            |
| Gly        | 554 ± 77     | 2.6 ± 6.7    |
| Ala        | 432 ± 122    | 11.0 ± 6.6   |
| Ser        | 360 ± 130    | 2.9 ± 3.4    |
| Glu        | 244 ± 43     | 2.7 ± 6.8    |
| Lys        | 131 ± 50     | 4.4 ± 5.7    |
| Val        | 110 ± 33     | 26.2 ± 4.1   |
| Thr        | 83 ± 20      | 0.0 ± 4.4    |
| Arg        | 67 ± 21      | 24.3 ± 8.0   |
| Asp        | 25 ± 8       | 9.9 ± 3.8    |
| Leu        | 20 ± 8       | 86.5 ± 4.9   |
| Ile        | 17 ± 7       | 7.4 ± 5.2    |
| Cys        | 10 ± 3       | 79.6 ± 6.3   |
| His        | 9 ± 2        | 61.6 ± 9.3   |
| Phe        | 6 ± 1        | 100.0 ± 0    |
| Met        | 5 ± 1        | 96.0 ± 1.7   |
| Tyr        | 2 ± 2        | 96.4 ± 1.4   |
| Trp        | 0 ± 0        | Not determined |

RESULTS

Free Amino Acids in Mouse Milk—The concentrations of free amino acids in mouse milk were determined (Table I, column I). Gly, Ala, Ser, and Glu were the most abundant species, and their concentrations were higher than 240 µM. Concentrations of Lys, Val, Thr, and Arg ranged from 67 to 131 µM. All other amino acids (Asp, Leu, Ile, Cys, His, Phe, Met, Tyr, and Trp) were lower than 25 µM. In particular, Met, Tyr, and Trp were less than 5 µM.

To examine the conversion of free amino acids in whey, amino acids added exogenously were incubated (Table I, column II). Gly, Ala, Ser, Glu, Lys, Thr, and Ile remained unchanged. Leu, Cys, His, Phe, Met, and Tyr decreased by greater than 60%, and among them, Met, Phe, and Tyr disappeared completely. The apparent decreasing order was Met, Phe, Tyr > Cys, Leu > His >> Arg, Val >> other amino acids. Amino acids with low contents in milk, shown in Table I (column I), were converted more efficiently and faster than other amino acids (r = -0.585, p = 0.014).

Characterization of LAO—As the marker of the LAO activity, the time-dependent production of H$_2$O$_2$ was measured (Fig. 1). LAO in the presence of L-Leu produced H$_2$O$_2$ in a time-dependent manner, while in the presence of L-His, L-Leu, or L-Phe, the production of H$_2$O$_2$ was no longer observed. LAO produced amino acids (Asp, Leu, Ile, Cys, His, Phe, Met, Tyr, and Trp) with low contents in milk, shown in Table I (column I), were deduced. LAO with the highest purity was obtained from fraction I and II. On the protein basis, LAO was eluted at three different fractions. The high LAO activity was found in fractions I and II. The apparent decreasing order was Met > Tyr > Leu, Cys > His >> other amino acids. During the incubation, the concentration of ammonia increased in a time-dependent manner (data not shown).

Purification of LAO—Skim milk was separated into the whey and casein fractions by ultracentrifugation. The whey fraction was used since it had the LAO activity. Gel filtration showed that the peak of LAO was detected at the position of apparent M, 113,000 (Fig. 3). LAO present in the 113-kDa fraction was further purified by ion-exchange chromatography (Fig. 4). LAO was eluted at three different fractions. The high LAO activity was found in fractions I and II. On the protein basis, LAO with the highest purity was obtained from fraction I. SDS-PAGE followed by silver staining showed that fraction I contained one protein species of M, 60,000 (Fig. 5).

Amino Acid Sequencing of LAO—LAO in fraction I was used for the amino acid sequencing analysis. The N-terminal amino acid sequence of LAO was LGYENLKVFQDPDFYEAFLLL. Amino acid sequences of lysylendopeptidase-digested peptides were LGYENLVK, TYVQK, NPGILGY, YRTDGPTSALHK, and ATRGHTAL. Among them, the peptide fragment ATRGHTAL ended at the last Leu and was identified as the C terminus of LAO.

Nucleotide Sequence of LAO cDNA—The nucleotide sequence of LAO cDNA is shown in Fig. 6a. The cDNA consisted of 1941 nucleotides plus poly(A) at the 3’-end. The N-terminal Leu was encoded at nucleotides 111–113. The C-terminal residue was Leu. The CTT codon was present at nucleotides 1599–1601, and coincidentally the TAG termination codon appeared at nucleotides 1602–1604. LAO was encoded at nucleotides 111–1601. The first ATG as the translation start codon was present at nucleotides 33–35. The signal peptide was encoded at nucleotides 33–110. The AATAAA polyadenylation signal was at nucleotides 1643–1648, and the poly(A) tail was present at nucleotide 1942. The amino acid sequence deduced from the cDNA is shown in Fig. 6b. The amino acid sequences, determined by the amino acid sequencing analysis, were found at positions 27–32, 27–46, 124–128, 160–166, 300–311, and 516–523 of deduced LAO. LAO and its signal peptide consisted of 497 and 26 amino acids, respectively.

Expression of LAO mRNA in the Mammary Gland—To examine the tissue- and stage-dependent expression of LAO, the level of LAO mRNA was determined by Northern blot analysis. Total RNA extracted from pregnant mice on day 18 was analyzed (Fig. 7). LAO mRNA in the mammary gland was detected.
as a clear band with a length of about 2.0 kb, and the faint band, probably corresponding to hnRNA, was present at the position of about 4.6 kb (Fig. 7a).

Except in the mammary gland, no expression was found in the brain, heart, liver, lung, muscle, and placenta (Fig. 7b).

The expression of LAO mRNA was determined in the mammary glands at various stages of pregnancy and lactation (Fig. 8). The expression of LAO mRNA was not detected on day 7 of pregnancy, but the faint band was observed on day 13 of pregnancy. LAO mRNA clearly increased on day 18 of pregnancy (1 day before parturition) compared with that on day 16 of pregnancy. LAO mRNA was expressed throughout lactation, but its expression was weak at the end of lactation.

The expression of LAO mRNA was examined by in situ hybridization (Fig. 9). On day 13 of pregnancy, no signal was detected with either antisense (Fig. 9a, antisense) or sense probes (Fig. 9b). On day 16 of pregnancy (Fig. 9c, antisense), some of the mammary epithelial cells showed a strong signal, but most epithelial cells had a weak or no positive signal. On day 18 of pregnancy (Fig. 9f, antisense), all of the mammary epithelial cells showed a strong and positive signal.

### Hormonal Control of the LAO Gene Expression

The alveolus had a narrow lumen on day 13 of pregnancy (Fig. 9, a and b). At 16 h after ovariectomy, the lumen expanded clearly, and numerous milk fat droplets appeared in the mammary gland (Fig. 9, c and d). LAO mRNA increased clearly between 8 and 16 h, peaked at around 24 h, and then decreased considerably (Fig. 10). In situ hybridization (Fig. 9c, antisense) showed that all of the mammary cells expressed the LAO mRNA, the expression pattern being very similar to that seen in the mammary gland on day 18 of pregnancy (Fig. 9f).

To further determine the progesterone- and/or glucocorticoid-dependent regulation of the LAO gene expression, bilateral ovaries and adrenal glands were removed on day 13 of pregnancy. LAO mRNA was not detected in the ovari-/adrenalectomized mouse mammary gland compared with that in the ovariectomized control. Administration of cortisol increased LAO mRNA, while progesterone showed no effect (Fig. 11).

### DISCUSSION

The cDNA encoding mouse milk LAO has a nucleotide sequence similarity of 53.2, 52.7, and 51.1% with cDNAs for snake venom Apoxin 1 (GenBank accession number AF093248), snake venom LAO (GenBank accession number AF071564), and mouse Fig1 protein (GenBank accession number U70429), respectively, as aligned by the Cluster method using the DDBJ program. Recently a cDNA with 508 bp was cloned from the lactating mouse mammary gland (GenBank accession number BE850855). The same nucleotide sequence is found in the mouse milk LAO cDNA at nucleotides 1252–1759. FAD is essential in expression of the LAO activity (22, 25). The nucleotide sequence encoding the FAD binding motif (10) was present at nucleotides 207–254.
ing lactation mimic those of γ-casein mRNA expression (26) and milk production in mice (27). In ovariectomized mid-pregnant mice, the mammary gland expresses the PRL receptor and casein genes in a time-dependent manner after the operation (28). We show here that the LAO gene is also expressed upon the onset of milk synthesis. In the circulating plasma, progesterone decreases and corticosterone increases at the end of pregnancy as well as after ovariectomy in mice (7). Glucocorticoid (cortisol or corticosterone) is essential to activate the PRL receptor gene (7) and to maintain the number of PRL receptors (23). Our data clearly show that the LAO gene expression is dependent upon cortisol and independent of progesterone. The similar hormone dependence is shown in the expression of the mitochondrial Tim23 gene in the mouse mammary gland (29). In the case of Neurospora crassa, LAO is an inducible enzyme, and its gene expression is under strong control (30, 31). Interleukin-4 induces the synthesis of Fig1 in the mouse B cell (10). It is thus concluded that glucocorticoid is essential for the LAO gene expression in the mammary gland.

Mouse milk LAO consisted of two subunits. Three distinct isoenzymes were detected, the electrically heterogeneous pattern being close to that seen in snake venom LAO (25, 32). The substrate specificity was close to that reported for other LAOs (11, 25). Unlike snake venom LAOs (12, 13, 33, 34), mouse milk LAO hardly oxidized L-Ile. Mouse milk LAO did not oxidize D-amino acids as seen in the rat liver and kidney (11). Similar to the other LAO (9, 35), mouse milk LAO produced H2O2 and ammonia. It is known that the snake venom LAO becomes inactivated by freezing (35, 36). After the prolonged storage at 20 or 50 °C, no decrease in the LAO activity was observed in mouse milk LAO. Similar results are shown in King cobra (Ophiophagus Hannah) venom LAO (37). The imbalance of free amino acids in milk is commonly observed among 15 different species examined (20), and we confirmed it in the mouse. Mouse milk LAO reacted with particular amino acids. We show here that amino acids convertible by LAO are few in mouse milk. Amino acids converted by mouse milk LAO could be classified further into fast-, intermediate- and slow-reactive species. It is expected that the concentration of H2O2 in milk is kept high and constant.

It has been shown that LAO in snake venom has potent antibacterial properties associated with the LAO activity (38). H2O2-induced DNA damage and cell death have been attributed to the direct cytotoxicity of H2O2 and other reactive oxygen species produced from H2O2 (39, 40). Lactoperoxidase is present in bovine milk (41). Together with hydrogen peroxide and SCN−, lactoperoxidase shows the antibacterial effect (42). H2O2 with peroxidase also shows the antibacterial effect (43). As shown here, LAO in the presence of free amino acids acts as an actual supplier of H2O2. Because some amino acids are consumed by LAO in the mammary gland, there is no doubt that their quantities observed at milking are not parallel with those actually secreted into milk. LAO in whey still produced H2O2 by utilizing endogenous free amino acids, the quantity of H2O2 being almost comparable to that produced in the presence of L-Leu at 200 nmol/ml. LAO produces one molecule of H2O2 through the oxidation of one amino acid (11). We speculate that

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**FIG. 6.** The nucleotide sequence of LAO cDNA (a) and deduced amino acid sequence of LAO (b). The nucleotide and amino acid sequences of the signal peptide are underlined in panels a and b, respectively.

**FIG. 7.** Tissue-specific expression of LAO mRNA. Tissues were collected from the same animal on day 18 of pregnancy. a, Northern blot of mammary gland RNA. The positions of RNA size markers are indicated. b, Northern blot (upper panel) and ethidium bromide staining (lower panel).

**FIG. 8.** LAO mRNA in the mammary gland during pregnancy and lactation. A Northern blot (upper panel) and ethidium bromide staining (lower panel) are shown. d, day.
the total amount of $\text{H}_2\text{O}_2$ produced in the milk of the gland is unexpectedly large, probably close to $\frac{1}{20}$ mol/ml. A high concentration of $\text{H}_2\text{O}_2$ is also reported in eye humors of the rabbit (44).

We present evidence showing that the lactating mouse mammary gland has a system capable of producing $\text{H}_2\text{O}_2$ constantly. As the production of $\text{H}_2\text{O}_2$ is a critical step to activate the antibacterial system (42), most bacteria do not survive in the mammary gland. Milk must be stored at low temperatures after milking. For this reason, it is possible to interpret that LAO is unable to produce $\text{H}_2\text{O}_2$ since free amino acids are no longer supplied from outside during storage. Occasionally, however, bacteria grow in the mammary gland. Mastitis is the most frequent worldwide disease in the dairy industry and a major cause of economic loss. It is an inflammatory reaction that most frequently develops in response to an intramammary bacterial infection (45, 46). This reaction often results in irreversible damages to the mammary epithelium even after the successful treatment and in permanent reduction in milk production (47). In the United States alone, economic losses are estimated at 2 billion dollars per year (48). In our preliminary

FIG. 9. In situ hybridization of LAO mRNA in the mammary gland during pregnancy. Mice (c and d) were ovariectomized on day 13 of pregnancy. a, day 13 (antisense); b, day 13 (sense); c, 16 h after ovariectomy (antisense); d, 16 h after ovariectomy (sense); e, day 16 (antisense); f, day 18 (antisense). The positive signal was brown-colored. The sections were counterstained with methylgreen. The original pictures were taken at the same magnification ($\times$200).

FIG. 10. Time-dependent expression of LAO mRNA in the mammary gland after ovariectomy. A Northern blot (upper panel) and ethidium bromide staining (lower panel) are shown.

FIG. 11. Cortisol-dependent expression of LAO mRNA. Ovariectomy (lanes 1 and 2) and ovari-/adrenalectomy (lanes 3–6) were performed. Sesame oil (lanes 1–3), progesterone (lane 4), cortisol (lane 5), and cortisol plus progesterone (lane 6) were injected at 0 h. The mammary gland was collected at 0 h (lane 1) and 16 h (lanes 2–6) after the injection. A Northern blot (upper panel) and ethidium bromide staining (lower panel) are shown.
experiments, the LAO activity of bovine milk was lower compared with that of mouse milk. We speculate that the production of H₂O₂ is insufficient to kill bacteria in dairy cows.

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