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A CNOX-like protein disulfide-thiol interchange activity of the cell surface of mouse sperm

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ABSTRACT Intact frozen mouse sperm were analyzed for the presence of ECTO-NOX-like protein disulfide-thiol interchange activity. Activity was determined both from the cleavage of a dithiodipyridine substrate and from the restoration of activity to scrambled and inactive ribonuclease. An activity was found using both methods of activity determination. The activity was resistant to inhibition by both capsaicin and bacitracin. The activity, which oscillated in the characteristic manner of ECTO-NOX proteins, was characterized by a pattern of five maxima and an overall period length of 24 min. Three of the five maxima were separated by an interval of 6 min and the remaining maxima were separated by intervals of 4.5 min to generate the repeating pattern with a period length of 24 min. The activity pattern was unusual in that all five of the maxima within the 24 min repeat were of approximately equal specific activity. Normally, for somatic cells, the first two maxima are involved in NADH oxidation and less pronounced in terms of protein disulfide-thiol interchange than are the remaining three.
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KEY WORDS

mouse sperm
protein disulfide isomerase
ECTO-NOX
NADH oxidase

There have been reports of protein disulfide isomerase associated with plasma membranes of several cell types (Kroning et al. 1994) and multiple functions have been attributed to the protein (Zair et al. 1999). Protein disulfide isomerase, for example, assists protein folding by expressing both an isomerase and a chaperone-like activity (Wang 1998; Chen et al. 1999). A protein disulfide isomerase-like activity has been found associated with the plasma membranes of sperm (Bohring et al. 2001) and has been suggested to be important for capacitation and the acrosome reaction. A similar activity has been associated with the acrosome (Ohtani et al. 1993).

In the present report a protein disulfide isomerase-like activity (protein disulfide thiol interchange) is demonstrated for the cell surface of mouse sperm based on assays using cell impermeant substrates. The activity has characteristics of a constitutive ECTO-NOX (CNOX) activity as is associated with plasma membranes of a variety of cells and tissues (Morré 1998) rather than that of a classical protein disulfide isomerase (e.g., ER 60) typically associated with the endoplasmic reticulum (Essex et al. 2001).

Methods

Frozen mouse sperm was supplied by Dr. Diego A. Ellerman, University of California, Davis, California (Ellerman et al. 2003).

Oxidation of NADH was determined from the disappearance of NADH measured at 340 nm in a reaction mixture containing 25 mM Tris-MES (pH 7.2), 100 μM GSH, 1 mM KCN to inhibit mitochondrial oxidase activity, and 150 μM NADH at 37°C with temperature control (± 0.5°C) and stirring (Morré and Morré 2003a, 2003b). Activities were measured using paired Hitachi U3210 spectrophotometers with continuous recording. Assays were initiated by addition of NADH. Assays were for 1 min and were repeated on the same sample every 1.5 min for the times indicated. A millimolar extinction coefficient of 6.22 was used to determine specific activity.

Protein disulfide-thiol interchange was determined spectrophotometrically from the activation of scrambled and inactive RNase with cleavage of cCMP measured spectrophotometrically as the assay (Lyles and Gilbert 1991) or from the cleavage of an artificial dithiodipyridine substrate (Morré et al. 1999).

Proteins were estimated by the bichinchoninic acid method (Smith et al. 1985). Bovine serum albumin was the standard.

Results

Mouse sperm exhibited a classical protein disulfide-thiol interchange (protein disulfide isomerase) activity as determined by the ability to activate scrambled and inactive ribonuclease (Fig. 1). cCMP was used as the substrate in a standard spectrophotometric assay (Lyles and Gilbert 1991). In contrast to a classical protein disulfide isomerase, the activation was not linear but periodic. Periods of activation were separated by periods of lesser activity at intervals of ca 24 min (Fig. 1, upper curve, arrows). In the absence of sperm, the scrambled ribonuclease remained inactive (Fig. 1, lower curve).

Assay of protein disulfide-thiol interchange using a dithiodipyridine (DTDP) substrate also revealed an oscil-

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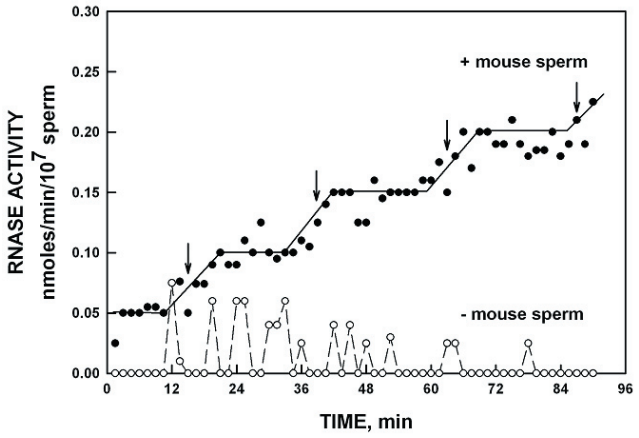

Figure 1. Protein disulfide-thiol interchange activity of mouse sperm measured from the activation of scrambled RNase. Preparations plus (upper curve) or minus (lower curve) sperm were assayed in parallel. The ribonuclease assay measured spectrophotometrically as the cleavage of cCMP (Lyles and Gilbert 1991).

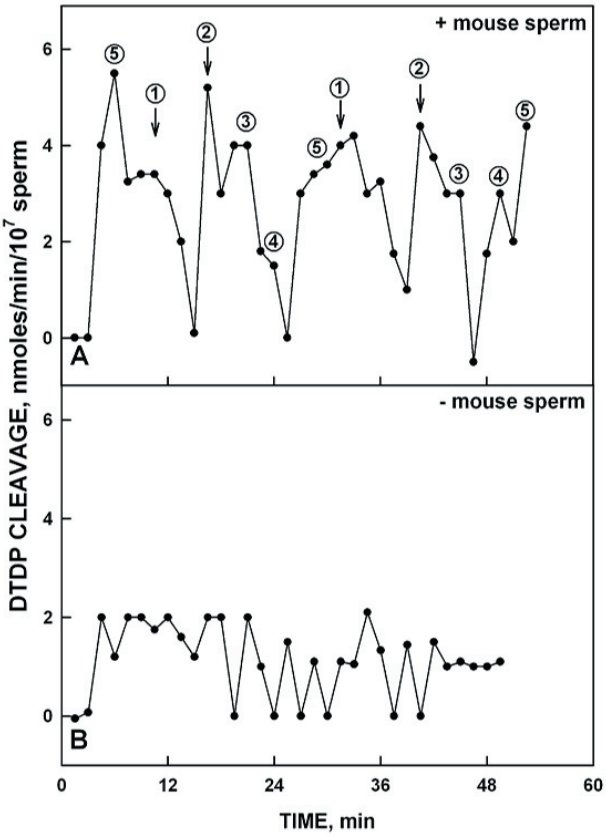

Figure 2. Rate of protein disulfide-thiol interchange of mouse sperm as determined by cleavage of dithiodipyridine (DTDP; Morré et al. 1999). Preparation plus (B) or minus (A) sperm were assayed in parallel.

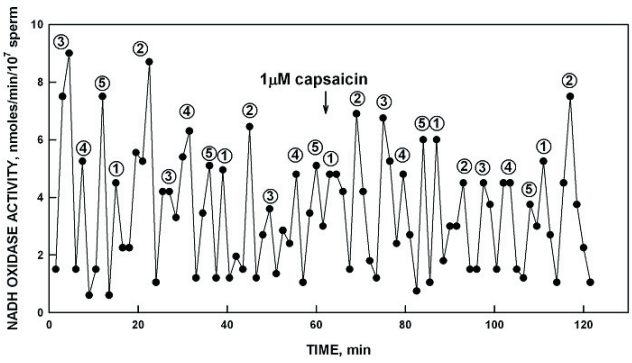

Figure 3. Rate of NADH oxidation by mouse sperm. The activity exhibited the typical CNOX activity pattern of oscillations with two maxima separated by intervals of 6 min and the remaining maxima separated by intervals of 4.5 min to generate a 24 min period (6 + 4 x 4.5). Capsaicin (1 μM) was added after 60 min.

lating pattern of activity also dependent upon the presence of sperm (Fig. 2B). Activity in the absence of sperm did not oscillate (Fig. 2A).

Since both the scrambled ribonuclease and the DTDP substrates revealed oscillatory patterns, the sperm were next assayed for the ability to oxidize NADH (Fig. 3). Again an oscillating pattern was observed consisting of five maxima, two of which ① and ② were separated by six min and three additional maxima ③, ④ and ⑤ were separated from each other and from the ① and ② doublet by intervals of 4.5 min to generate a period length of 24 min (6 + 4 X 4.5 min). Capsaicin added after 60 min was without effect on the activity as was bacitracin (not shown).

Parallel measurements of NADH oxidation (Fig. 4A) and DTDP cleavage (Fig. 4B) showed that both activities followed the same oscillatory pattern. These measurements were made simultaneously using two aliquots of the same sperm sample but with the two different substrates using paired spectrophotometers. With NADH oxidation, as in Figure 3, the 5 maxima of the 24 min repeating set of oscillations were of nearly equal magnitude (Fig. 4A). However, with the DTDP substrate to measure protein disulfide-thiol interchange (Fig. 4B), on average maxima labeled ③, ④ and ⑤ were somewhat greater than those labeled ① and ②.

Discussion

Mouse sperm exhibited a protein disulfide isomerase-like activity with characteristics more typical of that of constitutive ECTO-NOX proteins than that of a classical protein disulfide isomerase (Morré and Morré 2003a, 2003b). As is characteristic of ECTO-NOX proteins in general, their enzymatic activities oscillate in a characteristic pattern that imparts a time keeping attribute to the protein (Morré et al. 2002). ECTO-NOX proteins are located on the external cell surface, loosely bound, and exhibit both NADH (or

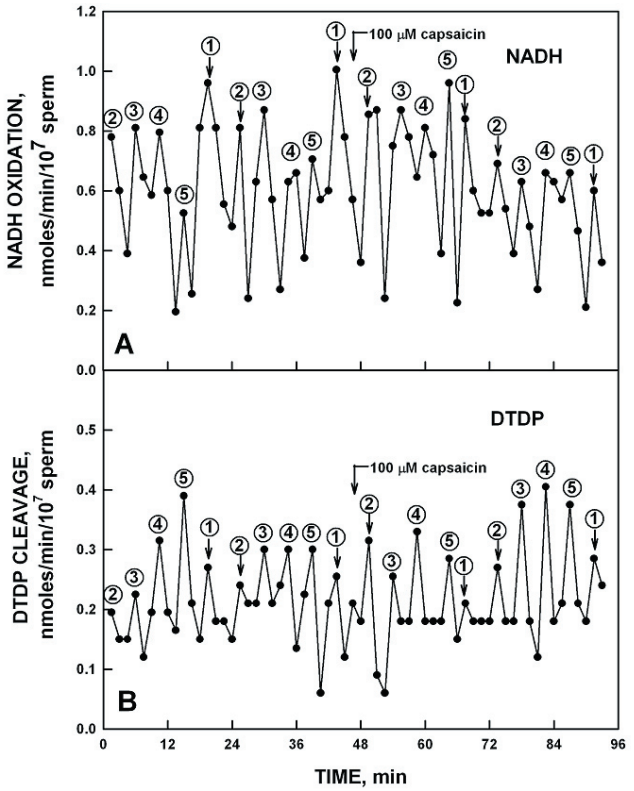

Figure 4. Rate of NADH oxidation (A) and rate of protein disulfide-thiol interchange (DTDP assay) (B) measured in parallel. Capsaicin (100 μM) was added after 45 min of incubation.

hydroquinone) oxidase activity and protein disulfide-thiol interchange. Normally the two activities alternate with two maxima separated by six min favoring NADH oxidation and the three maximum separated by 4.5 min favoring protein disulfide-thiol interchange.

The activity observed with the mouse sperm is unusual in that there is no distinction in activity between any of the maxima for NADH oxidation. They are all approximately equal. However, with activation of RNAse, periods of activity alternate with periods of inactivity as observed previously for both CNOX (Results unpublished) and a cancer-specific ECTO-NOX designated tNOX (Chueh et al. 2002). Similar results were observed in the experiments of Figure 4, where protein disulfide thiol interchange was augmented during the portion of the cycle attributed to maxima labeled ③, ④ and ⑤. These observations have mechanistic implications in that during this part of the cycle, the protein appears to have the capability, previously unobserved, of simultaneous oxidation of NADH and protein disulfide-thiol interchange.

tNOX differs from CNOX by being responsive to quinone site inhibitors such as capsaicin (Morré et al. 1995). Since the sperm activity was unaffected by capsaicin, it is an activity other than tNOX. Resistance to bacitracin distinguishes it from the more classical protein disulfide isomerases (Essex et al. 2001). The characteristic C-X-X-C motif common to most, if not all, members of the protein disulfide isomerase family of proteins (Ohnishi et al. 1995) is missing from tNOX. Neither tNOX nor CNOX appear to contain bound flavin nor are their activities dependent upon addition of flavins (FAD or FMN). The protein disulfide thiol interchange catalyzed by mouse sperm most clearly fit the category of a non-flavin-containing ECTO-NOX with functions in time keeping and physical membrane displacement rather than having the traditional chaperone function attributed to the protein disulfide isomerases of the endoplasmic reticulum, for example.

References

Bohring C, Krause E, Habermann B, Krause W (2001) Isolation and identification of sperm membrane antigens recognized by antisperm antibodies, and their possible role in immunological infertility disease. Mol Hum Reprod 7:113-118.

Chen J, Song JL, Zhang S, Wang Y, Cui DF, Wang CC (1999) Chaperone activity of DsbC. J Biol Chem 274:19601-19605.

Chueh P-J, Kim C, Cho NM, Morré DM, Morré DJ (2002) Molecular cloning and characterization of a tumor-associated, growth-related, and time-keeping hydroquinone (NADH) oxidase (tNOX) of the HeLa cell surface. Biochemistry 41:3732-3741.

Ellerman DA, Ha C, Primakoff P, Myles DG, Dveksler GS (2003) Direct binding of the ligand PSG17 to CD9 requires a CD9 site essential for sperm-egg fusion. Mol Biol Cell 14:5998-5103.

Essex DW, Li M, Miller A, Feinman RD (2001) Protein disulfide isomerase and sulfhydryl-dependent pathways in platelet activation. Biochemistry 40:6070-6075.

Kroning H, Kahne T, Ittenson A, Ansorge S (1994) Thiol-protein disulfide-oxireductase (protein disulfide isomerase), a new plasma membrane constituent of mature human B lymphocytes. Scand J Immunol 39:346-350.

Lyles MM, Gilbert HF (1991) Catalysis of the oxidative foliding of ribonuclease A by protein disulfide isomerase dependence of the rate on the composition of the redox buffer. Biochemistry 30:613-619.

Morré DJ (1998) NADH oxidase: A multifunctional ectoprotein of the eukaryotic cell surface. In Plasma Membrane Redox Systems and their Role in Biological Stress and Disease. Dordrecht, The Netherlands: Kluwer Academic Publishers, pp. 121-156.

Morré DJ, Morré DM (2003a) Spectroscopic analyses of oscillations in ECTO-NOX-catalyzed oxidation of NADH. Nonlinearity Biol Tox Med 1:345-362.

Morré DJ, Morré DM (2003b) Cell surface NADH oxidases (ECTO-NOX proteins) with roles in cancer, cellular time-keeping, growth, aging and neurodegenerative disease. Free Radical Res 37:795-808.

Morré DJ, Chueh PJ, Morré DM (1995) Capsaicin inhibits preferentially the NADH oxidase and growth of transformed cells in culture. Proc Natl Acad Sci USA 92:1831-1835.

Morré DJ, Chueh PJ, Pletcher J, Tang X, Wu LY, Morré DM (2002) Biochemical basis for the biological clock. Biochemistry 40:11941-199545.

Morré DJ, Gomez-Rey ML, Schramke C, Em O, Lawler J, Hobeck J, Morré DM (1999) Use of dipyridyl-dithio substrates to measure directly the protein disulfide-thiol interchange activity of the auxin stimulated NADH: protein disulfide reductase of soybean plasma membranes. Mol Cell Biochem 200:7-13.

Ohnishi K, Niimura Y, Hidaka M, Masaki H, Suzuki H, Uozumi T, Nishino T (1995) Role of cysteine 337 and cysteine 340 in flavoprotein that functions as NADH oxidase from Amphibacillus xylanus studied by site-directed mutagenesis. J Biol Chem 270:5812-5817.

Ohtani H, Wakui H, Ishino T, Komatsuda A, Miura AB (1993) An isoform of protein disulfide isomerase is expressed in the developing acrosome of

spermatids during rat spermiogenesis and is transported into the nucleus of mature spermatids and epididymal spermatozoa. Histochemistry 100:423-429.

Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC (1985) Measurement of protein using bicinchoninic acid. Anal Biochem 150:70-76.

Wang CC (1998) Protein disulfide isomerase assists protein folding as both an isomerase and a chaperone. Ann NY Acad Sci 864:9-13.

Zai A, Rudd MA, Scribner AW, Loscalzo J (199) Cell-surface protein disulfide isomerase catalyzes transnitrosation and regulates intracellular transfer of nitric oxide. Clin Invest 103:393-399.