Monoamines have been known to modify the cyclase activity in different invertebrate phyla, too[1,5,8-10]. Proctolin is the first identified peptide transmitter which has been shown to activate adenylate cyclase in the insect brain. Proctolin also caused an increase in the heartbeat rate of *Periplaneta americana* at concentrations below $10^{-9}$ M[11]. The adenylate cyclase of *Locusta* brain can be activated by monoamines, too; among them octopamine proved to be the most effective[5]. Comparing cyclase activation caused by monoamines or proctolin, it became obvious that proctolin had lower potency; however, sensitivity increased during development for both types of substances. The failure to activate guanosine cyclase by proctolin suggested the presence of separated receptor sites for these enzymes.

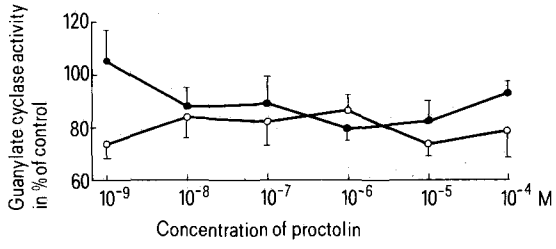


Fig. 2. Effect of proctolin on the guanylate cyclase activity. Each point is the mean ± SD of 4 determinations. Larvae: ●——●; control activity: 32.9 ± 4.1 pmoles cGMP/mg prot./min. Adult: ○——○; control activity: 41.7 ± 4.8 pmoles cGMP/mg prot./min.

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## Stereospecificity of hydrogen transfer of aldehyde reductase

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*Summary.* Aldehyde reductase from human liver catalyzes the hydrogen transfer from the pro-4R position on the dihydronicotinamide ring of the coenzyme to the *re* face of the carbonyl carbon atom of the substrate.

Aldehyde reductase from human liver is an NADPH-dependent monomeric oxidoreductase of broad substrate specificity. Several of its physical and chemical properties have been examined, and the enzyme has been shown to bear similarities to other low molecular weight reductases as well as to some oligomeric dehydrogenases[1,2]. These studies suggested an evolutionary link between the monomeric aldehyde reductase and the oligomeric dehydrogenases. One of the most highly conserved characteristics of a given dehydrogenase is its stereospecificity, and it has been contemplated that studies on the stereospecificity may reveal common features among the dehydrogenases having the same stereospecificity. In this work we have determined the stereospecificity of human liver aldehyde reductase and have compared it with that of other aldehyde reductases as well as with that of the oligomeric alcohol dehydrogenase.

*Experimental procedure.* 4S-(4-$^3$H) NADPH (B-NADPH) was prepared enzymatically with hexokinase and glucose-6-phosphate dehydrogenase (Boehringer Mannheim, FRG) from (1-$^3$H) glucose (New England Nuclear, Dreieichen, FRG) and $NADP^+$. The reaction mixture consisted of 0.1 M Tris-Cl, pH 7.5, 0.33 mM D-(1-$^3$H) glucose (18 Ci/mmole), 0.3 mM $NADP^+$, 5.5 mM ATP, 6.5 mM $MgCl_2$, 5 units of glucose-6-phosphate dehydrogenase and 2 units of hexokinase. The progress of the reaction was monitored by the increase in absorbance at 340 nm. Upon completion of the reaction unlabeled NADPH was added and the mixture was diluted with water to a conductivity of 5 mS and applied to a column (20 × 2 cm) packed with DEAE-Sepharose equilibrated against 20 mM Tris-Cl pH 8.0. 4S-(4-$^3$H) NADPH was eluted with a gradient 0–400 mM NaCl in 20 mM Tris-Cl, pH 8.0.

(4-$^3$H) $NADP^+$ was obtained by stereospecific oxidation of 4S-(4-$^3$H) NADPH with alcohol dehydrogenase. The reaction mixture consisted of 0.01 M Na phosphate, pH 7.0, 0.25 mM 4S-(4-$^3$H) NADPH, 2.5 mM 4-nitrobenzaldehyde and 20 units of horse liver alcohol dehydrogenase (Boehrin-

Stereospecificity of hydrogen transfer reactions of aldehyde reductase

| Reaction observed | | | Specific activity Donor | Acceptor | Percent transferred |
|---|---|---|---|---|---|
| | | | cpm/μmole | cpm/μmole | % |
| 4S-(4-$^3$H) NADPH | AR → | $NADP^+$ | $3.61 \cdot 10^6$ | $3.31 \cdot 10^6$ | 92 |
| 4R-(4-$^3$H) NADPH | AR → | $NADP^+$ | $7.84 \cdot 10^4$ | $0.016 \cdot 10^4$ | 0.2 |
| 4R-(4-$^3$H) NADPH | AR → | 4-nitrobenzylalcohol | $7.84 \cdot 10^4$ | $8.0 \cdot 10^4$ | 102 |
| (1-$^3$H) n-butanol | ADH → | NADH | $4.21 \cdot 10^3$ | $3.86 \cdot 10^3$ | 92 |

Tritium labeled coenzyme or substrate was incubated in the presence of the enzyme indicated and the corresponding product was isolated from the reaction mixture as outlined in the experimental section. Aliquots of 50 and 100 μl, respectively were mixed with 15 ml Unisolve (Koch Light Laboratories Ltd, Colnbrook, England) and counted for radioactivity. AR, Aldehyde reductase; ADH, alcohol dehydrogenase.

ger). When no further decrease in absorbance at 340 nm was observed, the (4-$^3$H) $NADP^+$ was purified in a manner analogous to that used for 4S-(4-$^3$H) NADPH with the exception that 10 mM Na phosphate, pH 6.0, was used as buffer.

4R-(4-$^3$H) NADPH (A-NADPH) was obtained from (4-$^3$H) $NADP^+$ and unlabeled D-glucose using conditions otherwise identical with those used for the preparation of 4S-(4-$^3$H) NADPH.

The following procedure was used to determine which of the 2 hydrogen atoms, pro-4S or pro-4R, was transferred to the aldehyde substrate. Aldehyde reductase, purified by the procedure of Wermuth et al.[1], was incubated in a mixture containing 10 mM Na phosphate pH 7.0, 1 mM 4S-(4-$^3$H) NADPH or 4R-(4-$^3$H) NADPH and 1 mM 4-nitrobenzaldehyde. When no further decrease in absorbance at 340 nm was observed the reaction mixture was subjected to ion exchange chromatography under the same conditions used for the purification of (4-$^3$H) $NADP^+$.

(1-$^3H_1$) n-Butanol was prepared enzymatically with aldehyde reductase from n-butanal and 4R-(4-$^3$H) NADPH. An excess of butanal was reduced by the addition of $NaBH_4$ and the (1-$^3H_1$) butanol was extracted with ether. $H_2O$ was added and the ether evaporated. The concentration of butanol was determined enzymatically with alcohol dehydrogenase in the presence of an excess of $NAD^+$ and semicarbazide according to the method of Bücher and Redetzki[3]. When no further increase in absorbance at 340 nm occurred the reaction mixture was diluted with water to a conductivity of 5 mS and applied to DEAE-Sepharose equilibrated against 10 mM $NH_4HCO_3$, pH 8.0. Elution of the NADH was accomplished by a linear gradient 0-400 mM NaCl in 10 mM $NH_4HCO_3$.

*Results and discussion.* The results of our studies on the stereospecificity of hydrogen transfer catalyzed by human liver aldehyde reductase are summarized in the table. Most of the tritium label (92%) remained on the coenzyme when 4S-(4-$^3$H) NADPH was used for the reduction of the aldehyde substrate.

In contrast, in the presence of 4R-(4-$^3$H) NADPH less than 1 percent of the initial radioactivity was recovered in $NADP^+$ and essentially all of the label (102%) was transferred to 4-nitrobenzaldehyde. These results indicate that aldehyde reductase catalyzes the transfer of the pro-4R hydrogen atom of the dihydronicotinamide ring to the aldehyde substrate without exchange with protons of the medium and thus belongs to the A-side specific dehydrogenases.

In order to determine the stereospecificity of aldehyde reductase with respect to the addition of the hydrogen atom to the carbonyl carbon atom of the substrate, (1-$^3$H) n-butanol, synthesized from n-butanal and 4R-(4-$^3$H) NADPH in the presence of aldehyde reductase, was reoxidized with alcohol dehydrogenase. The results given in the table show that most of the label was transferred from (1-$^3$H) butanol to the coenzyme. This indicates the same stereospecificity of aldehyde reductase and alcohol dehydrogenase, which is known to add the hydrogen atom of the coenzyme to the *re* face of the carbonyl carbon atom of the substrate[4]. A similar stereospecificity has been reported for the recution of glyceraldehyde by aldehyde reductase from pig kidney cortex[5]. Aldose reductase, a monomeric dehydrogenase showing striking similarities to aldehyde reductase is also an A-side specific enzyme[6]. On the other hand, rabbit tissues contain an aldehyde reductase with a stereospecificity for the pro-4S hydrogen of NADPH[7,8]. Thus the determination of the stereospecificity distinguishes between aldehyde reductases and may reveal structural and mechanistic differences in a class of enzymes which are otherwise very similar in physical and kinetic properties.

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## Occurrence of mitochondrial monoamine oxidase in human semen

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*Summary.* Mitochondrial monoamine oxidase (MAO) was found in human semen, showing its $K_m$ and $V_{max}$ values of 91.7 μM and 290 pmoles/mg of protein/60 min, respectively, with kynuramine as substrate. A major part of the activity was due to type A MAO.

Amine oxidases in mammalian tissues can be classified into 2 groups[1]. One is a flavin-containing amine oxidase (EC 1.4.3.4), which is known to be located in mitochondrial membranes. The other is a copper- and pyridoxal-containing group (EC 1.4.3.6) including diamine oxidase, serum monoamine oxidase (MAO) and an amine oxidase in connective tissues. Although diamine oxidase was found in human semen[2,3], to our knowledge, the presence of mitochondrial MAO in human semen has never been reported. In the present study, we found mitochondrial MAO in human semen and characterized it briefly.

*Materials and methods.* Human semen was collected at the Department of Urology, Nagoya University Hospital. It was centrifuged at 1500 × g for 5 min. The resulting supernatant fraction was centrifuged at 18,000 × g for 20 min and the pellet was suspended in 0.25 M sucrose solution. The suspension was recentrifuged at 18,000 × g for 20 min and the pellet was resuspended in 0.1 M sodium phosphate buffer (pH 7.4). This was used as an enzyme source.

The determination of MAO activity with kynuramine as substrate was carried out fluorometrically by the method of Kraml[4] with a slight modification[5]. The concentration of