METHOD OF CONVERTING BIG ENDOTHELIN-1 TO ENDOTHELIN-1 WITH HUMAN APOLIPOPROTEIN B

This application is a Continuation-in-Part Division of application Ser. No. 08/308,543, filed on Sep. 21, 1994, now abandoned.

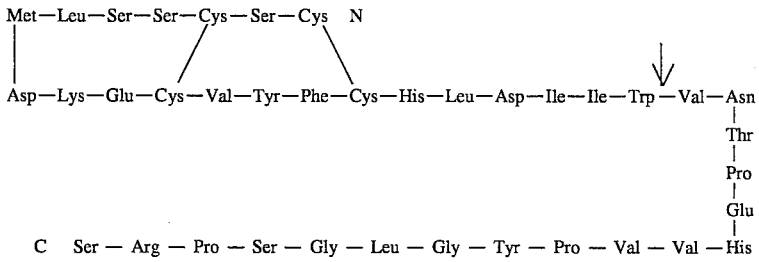
BACKGROUND OF THE INVENTION

This invention relates to an endothelin converting enzyme consisting of apolipoprotein B and more particularly to the use of apolipoprotein B as an endothelin converting enzyme.

Endothelin is an endothelial cell-derived vascular smooth muscle constricting factor discovered by Yanagisawa et al. in 1988, the presence of which has been identified in porcine, bovine and human or the like [M. Yanagisawa et al., Nature, Vol. 332, 411 (1988)].

Endothelin includes three types of isopeptides which are named endothelin-1, endothelin-2 and endothelin-3, respectively. Of these isopeptides, endothelin-1 has been confirmed to show the highest activity in human body. Endothelin, having strong and lasting action of constricting vascular smooth muscle cell and trachea, induces hypertension and constriction of respiratory tract and also induces at a high concentration (about 1–50 pmol/ml in blood level) ischemic cerebral and cardiac diseases such as cerebral apoplexy, strenocardia, myocardial infarction, cardiac incompetence and arrhythmia, nephropathy such as nephritis, circulatory failure of lung, liver and intestine, and asthma, thus sometimes bringing animals to the death.

Endothelin-1 is a 21-amino acid peptide which is produced by cleaving its precursor peptide, big endothelin-1 of the formula (SEQ ID NO: 1:



with the endothelin converting enzyme at the bond between the tryptophane residue at the 21st position from the N-terminus and the valine residue at the 22nd position from the N-terminus (shown by the downward arrow). This hydrolysis process is considered to be essential for production of endothelin-1 in vivo. Endothelin converting enzymes of bovine origin are those derived from cultured bovine endothelial cells or the like [K. Okada et al., Biochemical and Biophysical Research Communications, 171, No. 3, 1192 (1990)] and those derived from bovine adrenal medulla [T. Sawamura et al., Biochemical and Biophysical Research Communications, 168, No. 3, 1230 (1990)]. Regarding endothelin converting enzymes of human origin, WO 92/13944 discloses those prepared from human lung.

Endothelin having remarkable physiological activities as described above is produced enzymatically from its precursor i.e. big endothelin. Therefore, the elucidation of the endothelin converting enzyme will provide a means for inhibiting the production of endothelin in vivo. In addition,

this endothelin converting enzyme is expected to provide a useful reagent for analyzing the mechanism of vasoconstriction in vivo and for studying various diseases induced by endothelin.

Further, the elucidation of the endothelin converting enzyme (ECE) will provide an effective tool for searching and developing an ECE-inhibitor which would be clinically useful in the prophylaxis and treatment of various diseases (hyperendothelinemia) induced by hypersecretion of endothelin, such as hypertension, constriction of trachea, ischemic brain diseases and heart diseases, nephropathy, circulation failure of various organs (e.g., liver, lung, intestine, etc.), and asthma or the like.

Many reports were made on the relationship between atherosclerosis and endothelin. Atherosclerosis is a pathologic condition progressing by the abnormal proliferation of vascular smooth muscle cells. Further, Komuro, I. et al by FEBS Lett., 238, 249–252 (1988) have reported that ET-1 functions as a strong growth factor of vascular smooth muscle cells. Kanno, K. et al by JAMA, 264, 2868 (1990) have reported that patients with atherosclerosis exhibit significantly increased plasma levels of ET-1 as compared with healthy humans. Lerman, A. et al by N. Engl. J. Med. 325, 997–1001 (1991) have reported that plasma ET-1 concentrations show positive correlation with the extent of atherosclerotic vascular lesions.

Regarding the correlation between hyperlipemia which is the most common risk factor for atherosclerosis and endothelin, Horio, T. et al by Atherosclerosis, 89 (1991), 239–246 have reported that plasma ET-1 concentration in hypercholesterolemic rats increases significantly as compared with normal rats and also that there is positive correlation between plasma total cholesterol levels and plasma ET-1 concentration in hypercholesterolemic rats. Furthermore, it has been reported that plasma ET-1 concentration has positive correlation with plasma VLDL (very low density lipoprotein) concentration and plasma LDL (low density lipoprotein) concentration, whereas it does not correlate with plasma HDL (high density lipoprotein) concentration.

From the above reports, it is considered that elevated plasma ET-1 level by hyperlipemia functions as a growth factor of vascular smooth muscle cells which contributes to the progress of atherosclerosis.

However, it remains unknown why plasma ET-1 concentration will increase by hyperlipemia.

SUMMARY OF THE INVENTION

As a result of intensive investigations to prepare endothelin converting enzyme from human origin being easily available, we have found that apolipoprotein B of human origin has an endothelin-converting enzyme activity.

Since apolipoprotein B itself is ECE and one of the elements constituting VLDL and LDL of which the plasma