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ABSTRACT WITHDRAWN

EVALUATION OF NOVEL SELENOLTHIOL AND DITHIOL SEL-TAG VARIANTS FOR PURIFICATION AND ^{11}C-LABELING OF RECOMBINANT PROTEINS PRODUCED IN *E. COLI*

Q. Cheng,[1] L. Johansson,[1] A. Fredriksson,[2] J.-O. Thorell,[2] S. Stone-Elander,[2] E.S.J. Arner.[1]
[1]Medical Nobel Institute for Biochemistry, Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden; [2]Research Dept, Karolinska Pharmacy, Stockholm, Sweden.

Selenocysteine (Sec; U in one-letter code), the 21st amino acid existing in all selenoproteins, has unique biochemical properties due to its selenium atom, including a low pK_a and a high reactivity with many electrophilic agents. Mammalian thioredoxin reductase (TrxR) is a selenoprotein with a redox-active selenolthiol motif within its C-terminal tetrapeptide, -Gly-Cys-Sec-Gly-COOH (GCUG). We have used that motif as a "Sel-tag" for recombinant proteins produced in *Escherichia coli* and shown that it could be exploited for selenolate-targeted one-step purification as well as residue-specific fluorescent labeling with 4(5)-(iodoacetamido)fluorescein (5-IAF) and radiolabeling with either gamma emitters (^{75}Se) or positron-emitting radionuclides (^{11}C) (1).

We here extend these studies to other C-terminal tetrapeptide motifs, suggested by our prior results using mutants of *Drosophila melanogaster* TrxR. This enzyme has, instead of selenocysteine, a cysteine residue which is activated by flanking serine residues (2). In order to compare the reactivity of this type of tetrapeptide with our previous GCUG tag, we here produced recombinant Fel d 1, an allergen from the domestic cat (*Felis domesticus*), with two dithiol (-GCCG and -SCCS) and two selenolthiol (-GCUG and -SCUS) C-terminal tags. We subsequently assessed the use of these tags in one-step purification, fluorescent labeling and positron emitter labeling.

Phenylarsine oxide (PAO) columns could be utilized to efficiently purify all four Fel d 1 variants from whole bacterial lysates in a single purification step. The protein obtained was apparently homogeneous, as judged by Coomassie-stained SDS-PAGE analysis. Yields were about 5-10 mg pure protein from 1 liter bacterial culture.

We next found that all four tags could be fluorescent labeled with 5-IAF, with -GCCG being the least reactive and -SCUS being the most easy to label. Finally, we assessed the use of both the selenolthiol and dithiol tags for residue-selective labeling with a commonly used positron-emitting electrophilic precursor, [^{11}C]CH_3I. Similar to the fluorescent labeling, both the selenolthiol and dithiol variants could be radiolabeled with ^{11}C by incubating with reduced protein at room temperature for 10-25 min. The labeling efficiency of the selenolthiol motifs were, however, about 2-3-fold higher than the dithiol variants.

In summary, these results illustrate that not only the Sel-tag but also derivatives thereof can be used for purification and residue-specific radiolabeling. The differences observed in the labeling efficiencies of the tags merit further study into the mechanisms behind their reactivities. The fact that this approach permits efficient labeling of proteins with even such a short-lived tracer as [^{11}C]CH_3I is very promising for its use in developing novel applications for proteins labeled with a range of positron-emitting nuclides.

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DIFFERENTIAL BRAIN UPTAKE OF THE D/L-ISOMERS OF ALANINE, SERINE, PROLINE AND CIS-4-[^{18}F]FLUOROPROLINE AS POTENTIAL TRACERS FOR PET

D. Bauer,[1,3] K. Hamacher,[2,3] K.J. Langen,[1,3] D. Pauleit,[1,3] H. Herzog,[1,3] H.H. Coenen.[2,3]
[1]Institut fur Medizin, Forschungszentrum Julich GmbH, Julich, Germany; [2]Institut fur Nuklearchemie, Forschungszentrum Julich GmbH, Julich, Germany; [3]Brain Imaging Centre West, Forschungszentrum Julich GmbH, Julich, Germany.

Generally, L-amino acids are preferably transported into mammalian cells compared to their D-isomers and only L-amino acids are incorporated into proteins. Previous studies, however, indicated that D-[^{3}H]proline is accumulated in the brain of mice after injection while L-[^{3}H]proline is not [1]. We investigated the differential uptake of the D- and L-isomers of [^{3}H]alanine, [^{3}H]proline and [^{3}H]serine in the rat brain, all of which are considered as modulators of neuroexicitatory neurotransmitter systems in the brain. Furthermore, the D/L isomers of the PET tracer cis-4-[^{18}F]fluoroproline (D-/L-cis-FPro) were tested in human subjects.

Thirty male Fisher CDF rats were injected intravenously with D- and L-isomers of [^{3}H]alanine, [^{3}H]serine, [^{3}H]proline and cis-FPro. One or two hours after tracer injection the animals were killed. The brains were removed immediately and frozen in 2-methylbutane at -50 °C. Brains were cut in coronal sections and evaluated by quantitative dual tracer autoradiography. After injection of D-cis-FPro the brains of 4 rats were homogenized, centrifuged and the radioactivity in the supernatant and the pellet analyzed. Moreover, the cerebral uptake of D-cis-FPro was studied in two human subjects by PET and compared to the results of a previous study using L-cis-FPro in patients with brain tumors.

The standardized uptake value (SUV) of D-[^{3}H]proline in the cerebral cortex of the rat 2 h after intravenous injection was 1.29 ± 0.27 ($n = 4$) versus 0.30 ± 0.14 ($n = 9$) for L-[^{3}H]proline ($p < 0.001$) and 3.05 ± 1.18 for D-cis-FPro ($n = 9$) versus 0.06 ± 0.01 ($n = 4$) for L-cis-FPro ($p < 0.01$). For [^{3}H]-D-serine the SUV was 1.51 ± 0.14 ($n = 5$) versus 1.02 ± 0.21 ($n = 5$) for [^{3}H]-L-serine ($p < 0.01$). Brain uptake of [^{3}H]alanine was poor for both isomers.

Analysis of the rat brain tissue after injection of D-cis-FPro revealed no radioactivity in the proteins but a relevant part in the form of L-trans-FPro indicating racemisation. The PET studies in two humans yielded a threefold higher uptake in normal brain tissue for D-cis-FPro (20 - 60 min postinjection) than for L-cis-FPro [2]. The Patlak analysis of tracer kinetics in the human brain and plasma in two subjects yielded a fourfold higher rate constant for unidirectional flow for D-cis-FPro than for L-cis-FPro.

In conclusion, D-serine, D-proline and D-cis-FPro exhibit a higher uptake in the brain than their L-isomers. Thus, in contrast to the general doctrine some D-amino acids are preferably transported across the BBB. The biological background of this observation remains to be elucidated. The use of the D-isomers of radiolabeled amino acids, as verified for cis-4-[^{18}F]fluoro-D-proline for PET diagnostics may represent an interesting new field for in vivo imaging of various brain diseases.

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