Labeling of monoclonal antibodies and larger peptides with radioactive arsenic isotopes

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Introduction: Antibodies (AB) and larger peptides play a vital role in cancer imaging and therapy. As the enrichment of antibodies in tumor tissue is a slow process, isotopes with a long physical half-life are necessary to follow their pharmacokinetics. Also, it is expected that the combination of anti-tumor effects of the antibody alone and a therapeutic isotope will act synergistically. Arsenic provides several useful isotopes: the β^+ emitters ^{72}As ($T_{1/2}$ = 26.0 h) and ^{74}As ($T_{1/2}$ = 17.77 d) and the β^- emitter ^{77}As ($T_{1/2}$ = 38.83 h). We developed a labelling method where the arsenic isotope attaches to free sulfhydryl groups on the antibody. To increase their number the antibody needed to be modified. As a direct method, the reduction of endogenous disulfides via Sn^{2+} was used, which was compared the indirect method of SATA (N-succinimidyl S-acetylthioacetate) modification, where free NH_2-groups are chemically modified to create free SH groups.

Experimental: Sn^{2+} reduction was performed as described in [1]. The SATA modification was performed according to the Pierce Protocol [2]. After purification of the AB with gel size exclusion chromatography, the AB was labelled with nca AsI_3 as a direct labelling agent in 4 ml of PBS. Labelling time was 30 minutes at T=35°C and pH=7.8. The optimized production of nca AsI_3 is described in [3]. As antibodies we used various monoclonal and chimeric IGgs and the anti-PS ch3G4.

Results and Discussion: Fig.1&2 show the reaction schemes for the SATA-modification and labelling, consisting of 4 steps: acetylation of the primay amine, deacetylation, labelling via thioester formation and hydrolysis.

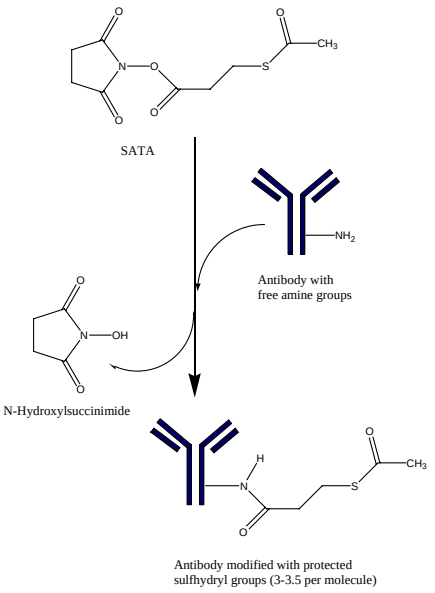

Fig. 1: SATA-Modification: reaction with the primary amine of an antibody

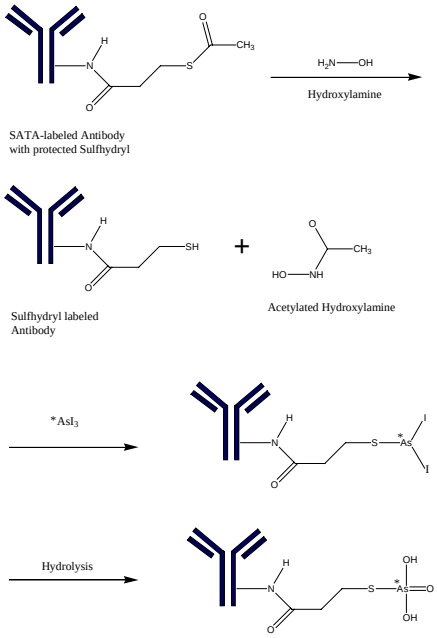

Fig. 2: SATA-Modification: deacetylation and labelling

Representative kinetics are shown in Fig. 3. for a human IGg. The final radiochemical yield reached with the SATA-modification is 95%, compared to about 85% for the Sn^{2+} reduction. Another disadvantage of the direct Sn^{2+} reduction is, that the opening of disulphur bonds could change the conformation and therefore the immunoreactivity of the AB. As shown in [3], this is not the case for the indirect method.

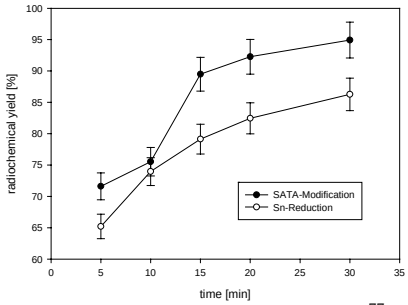

Fig. 3: Labelling kinetics for hu IGg with nca $^{77}AsI_3$

Conclusion: For future in vivo experiments, the SATA-modification to add additional free SH groups to antibodies for labelling with nca AsI_3 is recommended.

References:
[1] Gano, L. et al., Nuklearmedizin, **36**, 205-212, 1997
[2] Pierce Biotechnology, Technical Protocols, 2003
[3] Jennewein, M. et al., Annual Report, this issue.

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